American Journal of Analytical Chemistry, **2012, 3, 779-789**
doi:10.4236/ajac.2012.312104 Published Online December 2012 (http://www.SciRP.org/journal/ajac)

Acylhydrazide and Isatin Schiff Bases as Alternate UV-Laser Desorption Ionization (LDI) Matrices for Low Molecular Weight (LMW) Peptides Analysis

Syed Ghulam Musharraf[1,2*], Aisha Bibi[2], Najia Shahid[2], Muhammad Najam-ul-Haq[3], Momin Khan[2], Muhammad Taha[2], Uzma Rasool Mughal[2], Khalid Mohammed Khan[2]

[1]Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

[2] Hussain Ebrahim Jamal Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

[3]Department of Chemistry, Bahauddin Zakariya University, Multan, Pakistan

Email: *musharraf1977@yahoo.com

Received September 27, 2012; revised October 30, 2012; accepted November 12, 2012

ABSTRACT

Matrix-assisted laser desorption/ionization (MALDI) is a preferred and widely used mass spectrometric technique for the analysis of macromolecules. Limited UV-LDI matrices are available for the analysis of biomolecules due to the restricted structural features to serve in the laser desorption/ionization mechanism with a problem of background signals appearing in the low mass region. This paper describes the application of Schiff base derivatives of acylhydrazide and isatin as alternate UV-LDI matrices for the analysis of peptides with significantly low background signals. Thirty one compounds have been successfully employed as matrices for the analysis of low molecular weight (LMW) peptides (<2000 Da) including bradykinin and renin substrate tetra-decapeptide. Bovine serum albumin (BSA)-digest was also analyzed and identified through database search against Swiss-Prot by using MASCOT. The MS measurements were recorded by using dried droplet sample preparation procedures by mixing the matrix solution with analyte at a volume ratio of 1:2. Finally, LMW organic compounds (<500 Da) were also analyzed by the synthesized matrix materials which showed better S/N ratios and minimal background signals for low mass range in comparison to the comparable results with α-Cyano-4-hydroxycinnamic acid (HCCA), a preferred choice for peptide analysis.

Keywords: MALDI-MS; LDI Matrix; Acylhydrazide Schiff Bases; *Bis*-Schiff Bases of Isatin; Schiff Bases of Isatin; Peptides

1. Introduction

Soft ionization by matrix-assisted laser desorption/ionization mass spectrometry (MALDI) has become very important in mass spectrometric analysis, particularly, for biological macromolecules [1]. In the MALDI processes, laser and matrix play an effective role for the ionization of analyte. MALDI utilizes both UV and IR lasers as a source of energy [2], while UV lasers are widely used in various commercially available instruments. Commonly utilized UV lasers includes N_2 laser (337 nm) and frequency tripled and quadrupled Nd: YAG laser (355 nm and 266 nm, respectively). Moreover, smart beam, a modified UV lasers, has recently been introduced by the Bruker which provides significant enhancement in MALDI performance [3].

Likewise, different organic compounds have been screened for MALDI matrices. Initially, derivatives of benzoic acid, and related aromatic compounds were recognized as good MALDI matrices [4] for example, 2-(4-hydroxyphenylazo) benzoic acid, (HABA) for peptides, proteins and glycoproteins analysis [5] and 3-hydroxypicolinic acid (3-HPA) for oligonucleotides [6] were recognized as good UV-MALDI matrices. Compounds having functional groups other than carboxylic acid also have the tendency to be employed as MALDI matrices, for example, 3-aminoquinoline is a good matrix for polysaccharides and proteins [7], 2,4,6-trihydroxy acetophenone [8] and the laser dye coumarin 120 [9] has been found useful in MALDI-MS of oligonucleotides and monosulfated oligosaccharides, respectively. Fitzgerald *et al*. [10] introduced a number of basic matrices derived from substituted pyrimidines, pyridines, and anilines for the analysis of small proteins and nucleic acids. Recently, Y. Fukuyama *et al*. [11] have modified HCCA

*Corresponding author.

Copyright © 2012 SciRes.

by attaching C-8 alkyl chain which enables it to analyze hydrophobic peptides. Similarly, S. Martic *et al*. [12] have prepared allylated derivatives of DHB, HCCA and caffeic acid for the efficient analysis of compounds by MALDI-MS in low molecular weight region. Overall, efforts related to the different structural modifications of existing MALDI matrices or screening of new class of compounds as MALDI matrices are limited. However, more understanding of the structural correlation with mechanistic approach of matrices is required that may results in the development of better MALDI matrices with improved performance. Moreover, appearance of a large number of interfering matrix signals in the low *m*/*z* region is significant problem in MALDI MS analysis [13]. Different strategies have been published to analyze the analyte in the low mass region [14-27].

In this work, we have explored new classes of Schiff bases as alternate UV-LDI matrices for the analysis of low molecular weight peptides by MALDI-TOF-MS and to correlate the performance of a matrix with its chemical structure. The selection criteria of compounds was based on the structural features required for a MALDI matrix *i.e*. conjugated aromatic system, λ_{max} etc. A large number of Schiff base derivatives of acylhydrazide and isatin with necessary functionalities have been screened as potential matrices.

2. Experimental

2.1. Chemicals

Bradykinin (≥98%), renin substrate tetra-decapeptide (≥ 97%), insulin from bovine pancreas were purchased from Sigma-Aldrich (USA). Cholic acid (≥97%) was obtained from Wako (Japan) and chenodeoxycholic acid (≥97%) was obtained from MP Biomedical (Japan) while bovine serum albumin (BSA)-digest was purchased from Bruker Daltonics (Germany). All the solvents methanol (MeOH), trifluoroacetic acid (TFA), acetone ($(CH_3)_2CO$), and acetonitrile (ACN) were of HPLC grade and purchased from Sigma-Aldrich (USA). Deionized water (Milli-Q) was used during the study (Millipore, USA).

2.2. Synthesis of Matrix Materials

Acylhydrazide Schiff bases (Class I) were synthesized from acylhydrazides which were synthesized from different esters by refluxing with hydrazine hydrate for 2 h. Synthesized acylhydrazides were recrystallized in methanol. Acylhydrazide Schiff bases were prepared by condensing equimolar concentration of different acylhydrazide with different aromatic aldehydes and acetophenones by refluxing in ethanol for 3 to 4 h. The crude product was further recrystallized in methanol.

Bis-Schiff bases of isatin (Class II) were synthesized from the hydrazones which prepared by refluxing a mixture of isatin (1 g) and hydrazine hydrate (10 mL). The synthesized hydrazone (1 mmol) were refluxed with different substituted aromatic aldehydes (1 mmol) in methanol for 3 h. The progress of reaction was monitored by TLC. After cooling and filtration, the crystalline *bis*-Schiff bases were collected, washed with methanol and dried to afford compounds in high yields (>80%). Recrystallization from methanol resulted in pure crystals.

The Schiff bases of isatin (Class III) were synthesized by stirring a mixture of isatin (1 mmol) and different substituted aromatic amines (1 mmol) in small amount of water at room temperature for 20 - 30 h. The reaction was monitored by TLC. The yellow crystalline Schiff bases were collected by filtration, washed with water and dried to get pure crystals. Detail synthetic conditions and the spectroscopic data (^{1}H NMR) of synthesized Schiff bases of various classes have been reported earlier [28,29].

2.3. Preparation of Standard Solutions

Peptide standards (Bradykinin and renin substrate tetra-decapeptide) were prepared in 1mM concentrations in 0.1% TFA:ACN (1:1) and working standard solutions were prepared through the dilution of stock solution. BSA-digest and insulin from bovine pancreas were dissolved in 0.1% trifluoroacetic acid (TFA) in a concentration of 4 pmol/μL and 100 pmol, respectively. Cholic acid and chenodeoxycholic acid were prepared in methanol at the concentration of 0.1 μM.

2.4. UV-Vis Absorption Measurements

Schiff bases of acylhydrazide and bis-Schiff bases of isatin were dissolved in methanol, while Schiff bases of isatin were dissolved in acetone in a concentration range of 0.5 - 1.0 mM. The UV-Visible absorption maxima was recorded in the region of 200 - 800 nm by scanning against the reagent blank on a double beam UV/Visible spectrophotometer (Thermo Scientific Evolution 300, UK).

2.5. Mass Spectrometry

MALDI-MS measurements were carried out on Ultraflex III TOF/TOF (Bruker Daltonics, Bremen, Germany) mass spectrometer, equipped with a Smart beam laser (Nd: YAG, 355 nm) and an electrostatic reflector. The instrument was operated in reflector mode with the ion source 1 (ISI) set to 25.00 kV, source 2 (ISI) set to 21.50 kV, a lens voltage of 9.51 kV and laser energy was set between 50% - 60%. Flex analysis was used for the data analysis and the validation of data including the baseline subtracttion. External calibration was carried out by using pep-

Copyright © 2012 SciRes.

tide calibration standards (Bruker Daltonics, Bremen, Germany). 0.1% TFA:ACN (1:1) was used as a solvent to prepare solutions of different synthetic matrix materials, in a concentration range of 2 - 5 μg/μL. The solutions were sonicated for 5 minutes (Ultrasonic LC 38 H) and then centrifuged for 5 minutes (Centrifuge 5804 R Eppendorf). The supernatant was then used as matrix for the analysis. The dried droplet preparation method was used for the sample preparation by applying 1 μL saturated solution of different synthetic matrix materials with analyte at a volume ratio of 1:2 to a MALDI target plate and then allowing the droplet to dry at ambient conditions which formed a homogeneous film of the matrix. The dried co-crystallized samples were then analyzed by MALDI-MS.

Recorded BSA digest spectra with a newly developed matrices were submitted to the MASCOT search engine (Matrix Science, London, UK), using UniProt/Swiss-Prot (release July 2010, Homo sapiens, 18055 sequences) as the reference database. MASCOT search parameters were as follows: enzyme specificity trypsin, fixed modifications cysteine carbamidomethylation, variable modification methionine oxidation. The maximum number of missed cleavages was set to 3 and mass tolerance at (0.5 Da).

3. Results and Discussion

Schiff base derivatives of different class of compounds, including acylhydrazide Schiff bases (class I, nine compounds), *bis*-Schiff bases of isatin (class II, four compounds) and Schiff bases of isatin (class III, eighteen compounds) were screened as alternate matrices for the detection of low molecular weight peptides (**Table 1**).

3.1. Spectrophotometric Analysis of Synthesized Matrices

Wavelength maxima (λ_{max}) and the molar absorptivity (ε) of synthetic matrix materials were determined to verify their ability to absorb the laser energy at wavelength either matching or closer to the laser wavelength (355 nm) employed in UV-MALDI-MS (**Table 1**). Strong relationship was found between the signal intensities and UV spectrophotometric properties of the matrices. This observation is attributed to the spectral data obtained by various matrices having λ_{max} closer to the laser and has high value of the molar absorptivity (ε). However, desorption/ionization process is not exclusively dependent upon these two factors. The matrices based desorption/ionization mechanism is still not completely understood and other factors such as vacuum stability and solvent compatibility may also affect the performance of such matrices. The values of signal intensities and S/N ratio also indicate this anomaly (**Table 1**).

3.2. Screening of Schiff Base of Various Classes

Sample preparation method and solvent selection are two important parameters which played important role in achieving better signal to noise ratio (S/N) [30,31]. All screened synthetic compounds were prepared in 0.1% TFA:ACN for screening with matrix to analyte volume ratio of 1:2 for the detection of low molecular weight peptides (<2000 Da), bradykinin and renin substrate tetra-decapeptide.

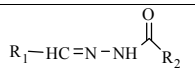
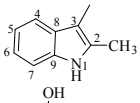
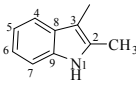
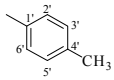
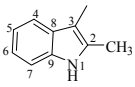
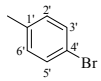
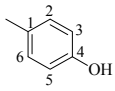
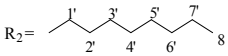
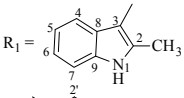
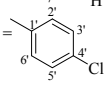
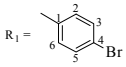
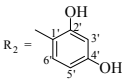
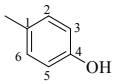
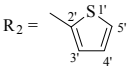
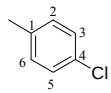
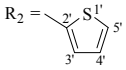
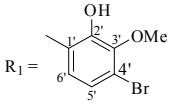
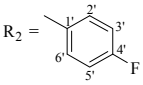
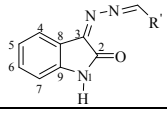
Nine compounds of class I (1 - 9) possessing λ_{max} between 304 - 339 nm were screened and found comparable to the existing MALDI matrix, HCCA in terms of intensity for the detection of peptides. Compounds 1, 2, 5 and 7 showed very high signal intensity ($>1 \times 10^5$) for peptides (**Table 1**). Four compounds of class II (10 - 13) possessing λ_{max} between 331 - 346 nm were screened as LDI matrix. All of them showed high intensity ($>1 \times 10^4$) of analyzed samples and were found suitable to be used as MALDI matrices for the analysis of low molecular weight peptides (**Table 1**). Similarly, eighteen compounds of class III (14 - 31), having λ_{max} between 326 - 416 nm, showed good signal intensity ($>1 \times 10^4$) of both peptides (**Table 1**). Overall, newly developed matrices produced excellent signal intensities of analytes with high reproducibility. Compounds 5, 10 and 31 when employed to analyze bradykinin peptide produced very high intensities with a standard deviation of ±0.15 (**Figure 1**), while the renin peptide analyzed with compounds 1, 10 and 31 afford similar results with a standard deviation of ±0.10 (**Figure 2**). However, almost no background signals were observed in the low mass region from these new matrices, hence, found suitable for the analysis of small molecules.

In all classes, a common chromophore was Schiff base while different aromatic substituents were attached to ty each class which enhanced both n-π^* and π-π^* transitions through electron lone pair on hetro atom and conjugated double bond system which produced the hyperchromic and bathochromic effects thus resulted in longer λ_{max} therefore, requires less energy for the excitation. This factor is directly related to the amount of laser power required to get a good intensity signal from a sweet spot on MALDI target.

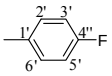
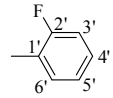
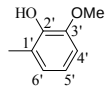
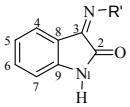
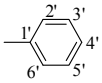
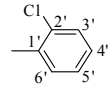
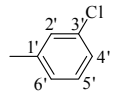
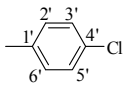
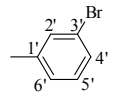
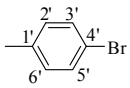
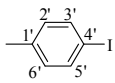
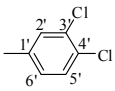
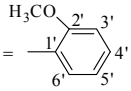
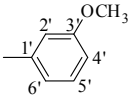
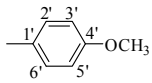
Moreover, among the all screened compounds, acylhydrazide Schiff bases (class I) showed excellent signal intensities. Therefore, compounds from class I were selected for further studies. BSA-digest sample was analyzed by using compounds of class I (1 - 7) to evaluate their ability to analyze peptides in complex biological samples. The spectrum showed promising signals of peptides (**Figure 3**) and the resulting mass spectrum were subjected to the database search against Swiss-Prot, using MASCOT for the identification. The sequence

Copyright © 2012 SciRes.

Table 1. The λ_{max} and molar absorptivities of different classes of compounds.

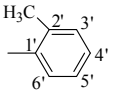
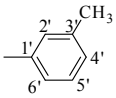
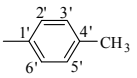
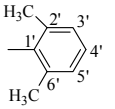
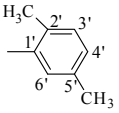
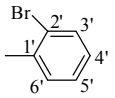
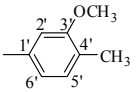
#	Matrix Classes	Structure	λ_{max} (nm)	ε^a	Log ε
	Class (I) Acylhydrazide Schiff bases				
1.	2-Hydroxy-*N*'-[(2-methyl-1*H*-indol-3-yl) methylene]benzohydrazide	R_1 =  R_2 =	315	16,274	4.2
2.	4-Methyl-*N*'-[(2-methyl-1*H*-indol-3-yl) methylene]benzohydrazide	R_1 =  R_2 = 	334	13,514	4.1
3.	4-Bromo-*N*'-[(2-methyl-1*H*-indol-3-yl) methylene]benzohydrazide	R_1 =  R_2 = 	338	20,900	4.3
4	N'-[(4-Hydroxyphenyl)methylene]nonano Hydrazide	R_1 =  R_2 = 	334	13,368	4.1
5.	4-Chloro-*N*'-[(2-methyl-1*H*-indol-3yl) methylene]benzohydrazide	R_1 =  R_2 = 	339	20,211	4.3
6.	4-Bromo-*N*'-[(4-hydroxyphenyl) methylene]benzohydrazide	R_1 =  R_2 = 	334	17,228	4.2
7.	*N*'-[(4-Hydroxyphenyl)methylene]-2-thiophene carbohydrazide	R_1 =  R_2 = 	337	16,358	4.2
8.	*N*'-[(4-Chlorophenyl)methylene]-2-thiophene carbohydrazide	R_1 =  R_2 = 	311	12,472	4.1
9.	4-Fluoro-*N*'-[(2-hydroxy-3-methoxyphenyl) methylene] benzohydrazide	R_1 =  R_2 = 	304	18,188	4.3
	Class (II) *Bis*-Shiff bases of isatin				

Continued

10.	2-Hydroxybenzaldehyde-N-(2-oxo-1, 2-dihydro-3H- indol-3-ylidene) hydrazone	R' =	334	8080	3.9
11.	4-Fluorobenzaldehyde-N-(2-oxo-1, 2-dihydro-3H-indol-3-ylidene) hydrazone	R' = 	331	9924	4.0
12.	2-Fluorobenzaldehyde-N-(2-oxo-1, 2-dihydro-3H-indol-3-ylidene) hydrazone	R' = 	331	5795	3.8
13.	2-Hydroxy-3-methoxy benzaldehyde-N-(2-oxo-1,2-dihydro-3H-indol-3-ylidene) hydrazone	R' = 	346	11,839	4.0
	Class (III) Schiff base of isatin				
14.	3-(Phenylimino)-1*H*-indol-2-one	R' = 	326	1186	3.1
15.	3-[(2-Chlorophenyl)imino]-1*H*-indol-2-one	R' = 	326	3040	3.5
16.	3-[(3-Chlorophenyl)imino]-1*H*-indol-2-one	R' = 	409	2181	3.3
17.	3-[(4-Chlorophenyl)imino]-1*H*-indol-2-one	R' = 	327	3527	3.5
18.	3-[(3-Bromophenyl)imino]-1*H*-indol-2-one	R' = 	327	2773	3.4
19.	3-[(4-Bromophenyl)imino]-1*H*-indol-2-one	R' = 	416	1228	3.1
20.	3-[(4-Iodophenyl)imino]-1*H*-indol-2-one	R' = 	327	4749	3.7
21.	3-[(3,4-Dichlorophenyl)imino]-1*H*-indol-2-one	R' = 	327	3610	3.6
22.	3-[(2-Methoxyphenyl)imino]-1*H*-indol-2-one	R' = 	327	2909	3.4
23.	3-[(3-Methoxyphenyl)imino]-1*H*-indol-2-one	R' = 	326	2724	3.4
24.	3-[(4-Methoxyphenyl)imino]-1*H*-indol-2-one	R' = 	328	3872	3.6

Copyright © 2012 SciRes.

Continued

25.	3-[(2-Methylphenyl)imino]-1*H*-indol-2-one	R' = 	326	2767	3.4
26.	3-[(3-Methylphenyl)imino]-1*H*-indol-2-one	R' = 	409	2560	3.4
27.	3-[(4-Methylphenyl)imino]-1*H*-indol-2-one	R' = 	327	3142	3.5
28.	3-[(2,6-Dimethylphenyl)imino]-1*H*-indol-2-one	R' = 	400	5389	3.7
29.	3-[(2,5-Dimethylphenyl)imino]-1*H*-indol-2-one	R' = 	327	6117	3.8
30.	3-[(2-Bromophenyl)imino]-1*H*-indol-2-one	R' = 	326	3348	3.5
31.	3-[(3-Methoxy-4-methylphenyl) imino]-1*H*-indol-2-one	R' = 	327	3635	3.6

a = **(L Mol^{-1}·cm^{-1})**

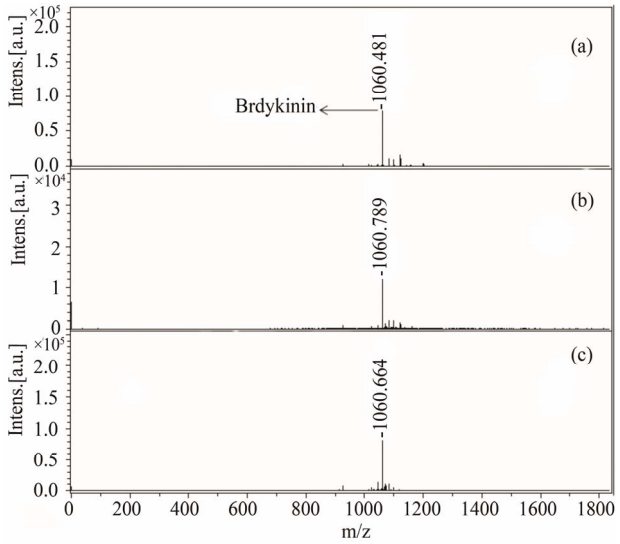


Figure 1. MALDI-TOF-MS spectra of a peptide bradykinin (50 pM) by applying different matrices (a) 5, (b) 10, and (c) 31. All bradykinin spectra were recorded in reflector mode by averaging 600 laser shots.

coverage of BSA using compound 1 was 44% (with average MASCOT score 105) and found to be very close with HCCA which gave 61% sequence coverage (with

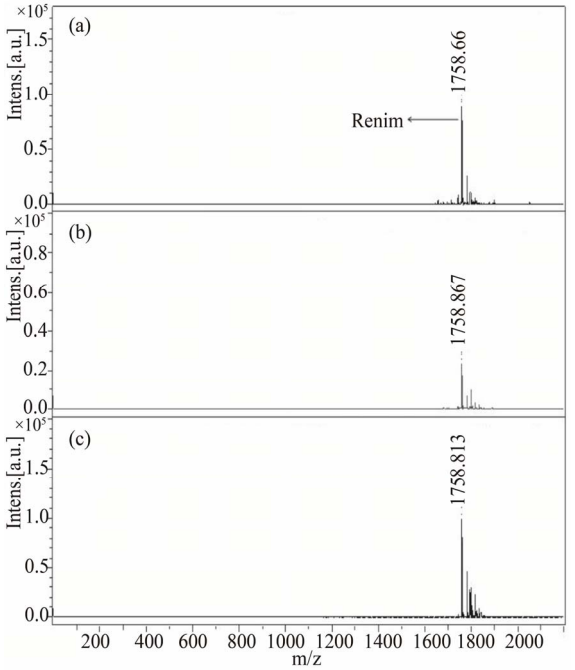


Figure 2. MALDI-TOF-MS spectra of a peptide rennin (50 pM) by applying different matrices (a) 1, (b) 10, and (c) 31. All rennin spectra were recorded in reflector mode by averaging 600 laser shots.

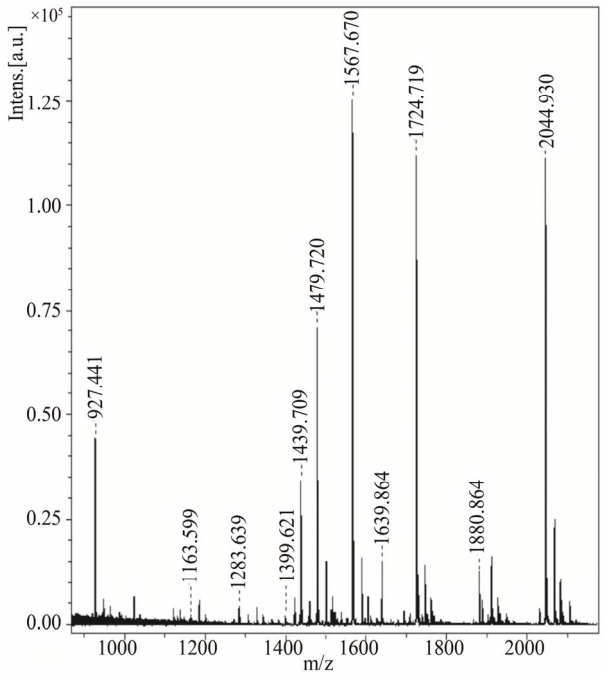


Figure 3. MALDI-TOF-MS spectra of BSA-digest with compound 3 as a matrix. BSA-digest (4 pmol/μL on target) spectra were recorded in reflector mode by averaging 600 laser shots.

average MASCOT score of 81), proving the ability to analyze peptides in complex biological samples (**Table 2**). Moreover, a protein, insulin (5733.492 Da) was also analyzed with compounds 1, 3 and 7 as an LDI matrix to observe the performance of these matrices in high mass region. These showed comparable results with HCCA (**Figure 4**) therefore, such compounds can also be employed to analyze proteins.

In order to evaluate the performance of new matrices in analyzing small molecules and to observe the background signals generated in low mass region, two low molecular weight compounds, cholic acid (408.57 Da) and chenodeoxycholic acid (392.57 Da) were analyzed. Cholic acid (0.1 μM) solution was analyzed with three different matrices (compounds 3, 13 and 31) showing excellent reproducibility with a standard deviation of only ±0.003, (**Figure 5**). Compound 3 (class I) was also used to analyze chenodeoxycholic acid which appeared at *m*/*z* 393.85 as $[M+H]^+$ while its potassium adduct $[M+K]^+$ appeared at *m*/*z* 431.13 (**Figure 6**). There was large number of interfering signals observed in lower mass range when analyzed with HCCA making it difficult to identify the chenodeoxycholic acid. Whereas the spectra of cholic acid and chenodeoxycholic acid recorded with the new matrices were very clean and nominal interfering signals were detected showing the ability of these new matrices to be used in the identification of metabolites.

Table 2. Data-base search results of BSA-digest, analyzed with various matrices.

Compounds	Average MASCOT Score	Average number of Peptides	% Sequence coverage
1	105	23	44
2	106	15	30
3	89	12	23
4	108	11	22
5	93	19	34
6	69	6	12
7	93	10	20
HCCA	81	35	61

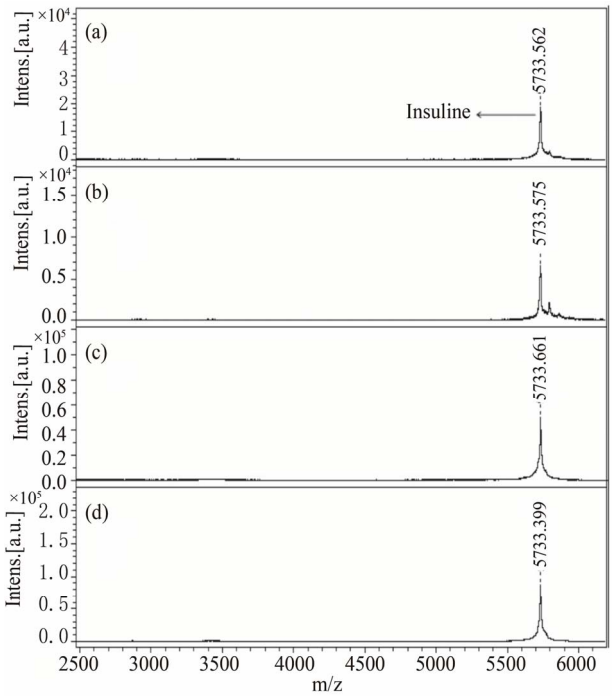


Figure 4. MALDI-TOF-MS spectra of a insulin (protein) by applying different matrices (a) 1, (b) 3 and (c) 7 and (d) HCCA. All insulin were recorded in reflector mode by averaging 600 laser shots.

The thickness of the co-crystallized analyte-matrix on MALDI plate is quite small, which offers an additional advantage of the newly developed matrices, as thicker films (especially observed in suspension-based matrix systems) cause shifting of the masses during MALDI experiments. Interestingly no mass shift was observed during the exact mass measurement in our experiments and the observed masses were found identical to their theoretical masses. Moreover, the newly synthetic matrices showed homogeneous co-crystallization with the

Copyright © 2012 SciRes.

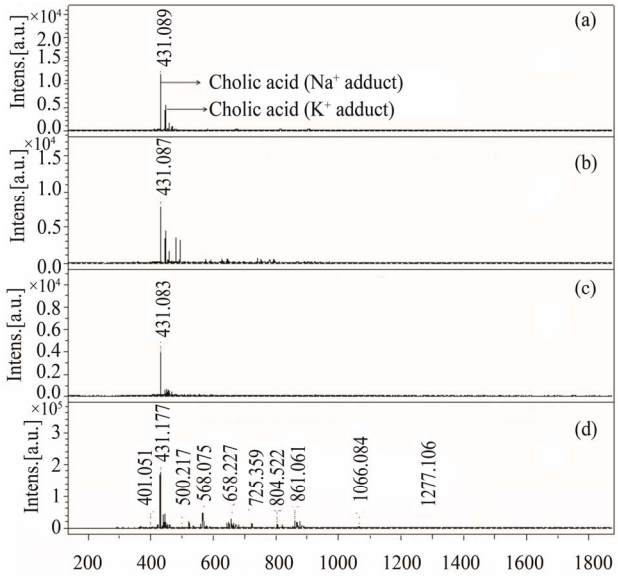


Figure 5. MALDI-TOF-MS spectra of cholic acid (0.1 μM) by applying different matrices (a) 3, (b) 13 (c) 31 and (d) HCCA. All cholic acid spectra were recorded in reflector mode by averaging 600 laser shots.

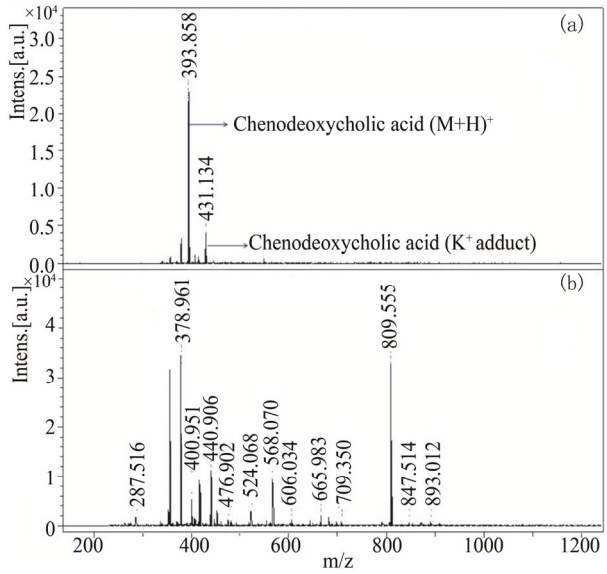


Figure 6. MALDI-TOF-MS spectra of chenodeoxycholic acid (0.1 μM) with compounds used as a matrix (a) 3 and (b) HCCA. All chenodeoxycholic acid spectra were recorded in reflector mode by averaging 600 laser shots.

analyte which is important to achieve the best spectral parameters (resolution, intensity and S/N ratio) especially when MALDI-MS spectra are recorded in an automatic mode.

4. Conclusion

We have reported here thirty one synthesized alternate matrices for the desorption and ionization of intact small peptides and compounds. Large number of Schiff bases of acylhydrazide, Schiff base and *bis*-Schiff bases of isatin with necessary functionalities have been screened. These LDI matrices have an ability to ionize the analyte molecules and act as energy absorbing materials at the employed laser wavelength with minimum background signals. As alternate LDI matrices, a number of conjugated aromatic systems in their structures ensure the laser energy transformations to the analytes. Furthermore, this approach lead to the development of structure-property relationship models, which in turn, could lead to a better understanding of the MALDI mechanism and the development and discovery of better matrices in future.

REFERENCES

[1] J. Gross and K. Strupat, "Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Applied to Biological Macromolecules," *Trends in Analytical Chemistry*, Vol. 17, No. 8-9, 1998, pp. 470-484. doi:10.1016/S0165-9936(98)00060-0

[2] D. J. Rousell, S. M. Dutta, M. W. Little and K. K. Murray, "Matrix-Free Infrared Soft Laser Desorption/Ionization," *Journal of Mass Spectrometry*, Vol. 39, No. 10, 2004, pp. 1182-1189. doi:10.1002/jms.706

[3] A. Holle, A. Haase, M. Kayser and J. Hohndorf, "Optimizing UV Laser Focus Profiles for Improved MALDI Performance," *Journal of Mass Spectrometry*, Vol. 41, No. 6, 2006, pp. 705-716.

[4] J. Krause, M. Stoeckli and U. P. Schlunegger, "Studies on the Selection of New Matrices for Ultraviolet Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry," *Rapid Communication in Mass Spectrometry*, Vol. 10, No. 15, 1996, pp. 1927-1933. doi:10.1002/(SICI)1097-0231(199612)10:15<1927::AID-RCM709>3.0.CO;2-V

[5] P. Juhasz, P. Costello and K. Biemann, "Matrix-Assisted Laser Desorption Ionization Mass Spectrometry with 2-(4-Hydroxyphenylazo) Benzoic Acid Matrix," *Journal of the American Society for Mass Spectrometry*, Vol. 4, No. 5, 1993, pp. 399-409. doi:10.1016/1044-0305(93)85005-I

[6] K. J. Wu, A. Steding and C. H. Becker, "Matrix-Assisted Laser Desorption Time-of-Flight Mass Spectrometry of Oligonucleotides Using 3-Hydroxypicolinic Acid as an Ultraviolet-Sensitive Matrix," *Rapid Communication in Mass Spectrometry*, Vol. 7, No. 2, 1993, pp. 142-146. doi:10.1002/rcm.1290070206

[7] J. O. Metzger, R. Woisch, W. Tuszynski and R. Angermann, "New Type of Matrix for Matrix-Assisted Laser Desorption Mass Spectrometry of Polysaccharides and Proteins," *Fresenius Journal of Analytical Chemistry*, Vol. 349, No. 6, 1994, pp. 473-474. doi:10.1007/BF00322937

[8] U. Pieles, W Zürcher, M Schär and H. Moser, "Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry: A Powerful Tool for the Mass and Sequence Analysis of Natural and Modified Oligonucleo-

tides," *Nucleic Acids Research*, Vol. 21, No. 14, pp. 3191-3196.

[9] Y. Dai, R. M. Whittal, C. A. Bridges, Y. Isogai, O. Hindsgaul and L. Li, "Matrix-Assisted Laser Desorption Ionization Mass Spectrometry for the Analysis of Monosulfated Oligosaccharides," *Carbohydrate Research*, Vol. 304, No. 1, 1997, pp. 1-9. doi:10.1016/S0008-6215(97)00195-X

[10] M. C. Fitzgerald, G. R. Parr and L. M. Smith, "Basic Matrices for the Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Proteins and Oligonucleotides," *Analytical Chemistry*, Vol. 65, No. 22, 1993, pp. 3204-3211. doi:10.1021/ac00070a007

[11] Y. Fukuyama, R. Tanimura, K. Maeda, M. Watanabe, S. Kawabata, S. Iwamoto, S. Izumi and K. Tanaka, "Alkylated Dihydroxybenzoic Acid as a MALDI Matrix Additive for Hydrophobic Peptide Analysis," *Analytical Chemistry*, Vol. 84, No. 9, 2012, pp. 4237-4243. doi:10.1021/ac300540r

[12] S. Martic, J. D. Brennan, M. A. Brook, S. Ackloo and N. Nagy, "Towards the Development of a Covalently Tethered MALDI System—A Study of Allyl-Modified MALDI Matrixes," *Canadian Journal of Chemistry*, Vol. 85, No. 1, 2007, pp. 66-76. doi:10.1139/v06-185

[13] Z. Guo, Q. Zhang , H. Zou, B. Guo and J. Ni, "A Method for the Analysis of Low-Mass Molecules by MALDI-TOF Mass Spectrometry," *Analytical Chemistry*, Vol. 74, No. 7, 2002, pp. 1637-1641. doi:10.1021/ac010979m

[14] G. McCombie and R. Knochenmuss, "Small-Molecule MALDI using the Matrix Suppression Effect to Reduce or Eliminate Matrix Background Interferences," *Analytical Chemistry*, Vol. 76, No. 17, 2004, pp. 4990-4997. doi:10.1021/ac049581r

[15] F. O. Ayorinde, P. Hambright, T. N. Porter and Q. L. Keith, "Use of Meso-Tetrakis (Pentafluorophenyl) Porphyrin as a Matrix for Low Molecular Weight Alkyl Phenol Ethoxylates in Laser Desorption/Ionization Time-of-Flight Mass Spectrometry," *Rapid Communication in Mass Spectrometry*, Vol. 13, No. 24, 1999, pp. 2474-2479. doi:10.1002/(SICI)1097-0231(19991230)13:24<2474::AID-RCM814>3.0.CO;2-0

[16] A. Tholey and E. Heinzle, "Ionic (Liquid) Matrices for Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry—Applications and Perspectives," *Analytical and Bioanalytical Chemistry*, Vol. 386, No. 1, 2006, pp. 24-37. doi:10.1007/s00216-006-0600-5

[17] D. W. Armstrong, L. Zhang, L. He and M. L. Gross, "Ionic Liquids as Matrixes for Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry," *Analytical Chemistry*, Vol. 73, No. 15, 2001, pp. 3679-3686. doi:10.1021/ac010259f

[18] M. Najam-ul-Haq, M. Rainer, C. W. Huck, P. Hausberger, H. Kraushaar and G. K. Bonn, "Nanostructured Diamond-Like Carbon on Digital Versatile Disc as a Matrix-Free Target for Laser Desorption/Ionization Mass Spectrometry," *Analytical Chemistry*, Vol. 80, No. 19, 2008, pp. 7467-7472.

[19] J. Wei, J. M. Buriak and G. Siuzdak, "Desorption-Ionizationmass Spectrometry on Porous Silicon," *Letters to Nature*, Vol. 399, No. 77, 1999, pp. 243-246. doi:10.1038/20400

[20] S. Zhang, Y. Chen, J. A. Liu, S. X. Xiong, G. H. Wang, J. Chen and G. Q. Yang, "New Matrix of MALDI-TOF MS for Analysis of Small Molecules," *Chinese Chemical Letters*, Vol. 20, No. 12, 2009, pp. 1495-1497. doi:10.1016/j.cclet.2009.06.031

[21] T. Kinumi, T. Saisu, M. Takayama and H. Niwa, "Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Using an Inorganic Particle Matrix for Small Molecule Analysis," *Journal of Mass Spectrometry*, Vol. 35, No. 3, 2000, pp. 417-422. doi:10.1002/(SICI)1096-9888(200003)35:3<417::AID-JMS952>3.0.CO;2-#

[22] S. Ren, L. Zhang, Z. Cheng and Y. Guo, "Immobilized Carbon Nanotubes as Matrix for MALDI-TOF-MS Analysis: Applications to Neutral Small Carbohydrates," *Journal of the American Society for Mass Spectrometry*, Vol. 16, No. 3, 2005, pp. 333-339. doi:10.1016/j.jasms.2004.11.017

[23] X. Dong, J. Cheng, J. Li and Y. Wang, "Graphene as a Novel Matrix for the Analysis of Small Molecules by MALDI-TOF MS," *Analytical Chemistry*, Vol. 82, No. 14, 2010, pp. 6208-6214. doi:10.1021/ac101022m

[24] H. Kim, J. Lee, S. Park, H. W. Ro, D. Y. Yoo and D. Y. Yoon, "Observation of Low Molecular Weight Poly (Methylsilsesquioxane)s by Graphite Plate Laser Desorption/Ionization Time-of-Flight Mass Spectrometry," *Analytical Chemistry*, Vol. 72, No. 22, 2000, pp. 5673-5678. doi:10.1021/ac0003899

[25] M. J. Dale, R. Knochenmuss and R. Zenobi, "Graphite/Liquid Mixed Matrices for Laser Desorption/Ionization Mass Spectrometry," *Analytical Chemistry*, Vol. 68, No. 19, 1996, pp. 3321-3329. doi:10.1021/ac960558i

[26] C. Black, C. Poile, J. Langley and J. Herniman , "The Use of Pencil Lead as a Matrix and Calibrant for Matrix-Assisted Laser Desorption/Ionization," *Rapid Communication in Mass Spectrometry*, Vol. 20, No. 7, 2006, pp. 1053-1060. doi:10.1002/rcm.2408

[27] G. J. Langley, J. M. Herniman and M. S. Townell, "2B Or Not 2B, That is the Question: Further Investigations into the Use of Pencil as a Matrix for Matrix-Assisted Laser Desorption/Ionization," *Rapid Communication in Mass Spectrometry*, Vol. 21, No. 2, 2007, pp. 180-190. doi:10.1002/rcm.2827

[28] K. M. Khan, M. Khan, M. Ali, M. Taha, S. Rasheed, S. Perveen and M. I. Choudhary, "Synthesis of Bis-Schiff Bases of Isatins and their Antiglycation Activity," *Bioorganic & Medicinal Chemistry*, Vol. 7, No. 22, 2009, pp. 7795-7801. doi:10.1016/j.bmc.2009.09.028

[29] K. M. Khan, U. R. Mughal, Samreen, S. Perveen and M. I. Choudhary, "Schiff Bases of Isatin: Potential Anti-Leishmanial Agents," *Letters in Drug Design & Discovery*, Vol. 5, No. 4, 2008, pp. 243-249. doi:10.2174/157018008784619915

[30] M. W. F. Neilen, "MALDI Time-of-Flight Mass Spectrometry of Synthetic Polymers." *Mass Spectrometry Reviews*, Vol. 18, No. 5, 1999, pp. 309-344. doi:10.1002/(SICI)1098-2787(1999)18:5<309::AID-MAS

Copyright © 2012 SciRes.

2>3.0.CO;2-L

[31] S. F. Macha, P. A. Limbach and P. J. Savickas, "Application of Nonpolar Matrices for the Analysis of Low Molecular Weight Nonpolar Synthetic Polymers by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry," *Journal of the American Society for Mass Spectrometry*, Vol. 11, No. 8, 2000, pp. 731-773. doi:10.1016/S1044-0305(00)00137-9

Copyright © 2012 SciRes.

Appendix

Table A1. Signal intensity and S/N ratio of synthetic matrix material with low molecular weight analytes.

Compounds	Bradykinin		Renin substrate tetra-decapeptide	
	S/N	Sig. Int.	S/N	Sig. Int.
1	1243	303,774	378	89,505
2	186	122,638	760	70,670
3	1078	59,705	702	55,347
4	513	79,804	191	34,052
5	1114	97,047	7151	82,709
6	246	73,297	192	34,052
7	892	1,268,674	1417	74,896
8	160	1687	236	10,335
9	951	58,606	834	95,126
10	352	12,917	643	88,445
11	992	126,386	250	59,958
12	706	83,512	648	53,400
13	344	69,710	356	60,418
14	523	62,378	423	23,762
15	204	40,104	236	41,150
16	292	73,000	362	38,828
17	497	77,183	426	33,273
18	1835	153,067	781	150,047
19	386	86,938	276	45,939
20	699	106,798	330	98,533
21	502	105,059	308	98,153
22	288	45,002	434	47,010
23	150	51,728	318	75,890
24	538	87,681	451	105,636
25	444	26,336	1015	110,685
26	509	86,173	681	79,172
27	736	58,769	297	59,142
28	330	56,341	800	21,265
29	347	64,819	375	131,250
30	214	19,307	265	18,867
31	171	62,204	357	102,072
HCCA	832	86,824	378	89,505

Copyright © 2012 SciRes.