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Identification of amino acids by material enhanced laser desorption/ionisation mass spectrometry (MELDI-MS) in positive- and negative-ion mode

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ABSTRACT

In the present study, different silica gel modifications were evaluated for their application as target surface for material enhanced laser desorption/ionisation mass spectrometric (MELDI-MS) investigation of amino acids. 4,4′-Azodianiline (ADA-silica) modified silica gel was successfully employed for the qualitative analysis of amino acids in positive- and in negative-ion mode. Further no derivatisation of amino acids was necessary, as the introduced system allowed the direct analysis of targets and delivered spectra with excellent signal intensity and signal-to-noise ratio within a few minutes. The influence of surface chemistry, ionisation mode and the nature of analytes on signal intensity was studied and discussed. Detection limit of 2.10 pg (10 fmol) was achieved by employing ADA-silica in positive-ion mode. Finally, xylem saps from different types of trees were analysed. This proved the high performance and excellent behaviour of the introduced target surface material.

1. Introduction

More than 300 amino acids have been described in nature. These compounds have great biochemical importance [1], especially the 20 common amino acids that are the basic units of proteins. The importance of amino acid analysis is obvious in many areas of science including clinical science, biochemistry, food science [2,3] and pharmacy [4–7].

In fact, a large number of analytical techniques have previously been utilised and described as shown in the extensive literature on the subject. Simple and highly sophisticated chromatographic and mass spectrometric techniques are cited alongside with amino acid analyser and voltametric titration. Generally, prior to analysis, amino acids are subjected to a derivatisation step, the type of which is selected according to different factors. In the case of liquid chromatography (LC) [5,8–14], amino acid analyser [15] or capillary electrophoresis [16–20] analytes have to be coupled to UV-absorbing units in order to enable UV-detection. For the gas chromatographic analysis, targets have to be silylated [21,22] or fluorinated [23], as vaporization is essential for separation and detection. Finally, especially for liquid chromatography hyphenated to mass spectrometry [24–26] derivatisation of analytes or considerable pre-treatment of samples like desalting is necessary in order to gain stable targets and, consequently, high detector signals. In general, derivatisation-based analysis may suffer from interference caused by the excess of reagents or reaction media, derivative instability, the inability of some reagents to derivatise the secondary amino acids and long preparation time [27,28]. Furthermore, several derivatives may be produced from single solute. This increases the complexity of the sample and makes its determination difficult. Likewise, pre-treatment process is not only time consuming, but every step leads to the loss of analyte. On the basis of these facts, it becomes clear that amino acid analysis is time consuming, cost intensive and loosing considerable amount of analyte, and, thus, bears negative implications for screening programs and projects with a high number of samples.

By contrast, matrix assisted laser desorption/ionisation-time of flight-mass spectrometry (MALDI-TOF-MS) can reach a high-automated throughput without previous derivatisation of analytes or pre-treatment of samples, delivering highly reliable qualitative information about the mass to charge ratio (m/z) of investigated amino acids. Since its introduction in 1987, MALDI-MS technique has mainly been utilized for the qualitative analysis of biomolecules like proteins and peptides. The application as screening technology for small metabolites like carbohydrates or amino acids is limited because of the interferences produced by employed matrix materials and because of problems associated with heterogeneity of analyte crystallization and control of ion suppression effect [29].

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However, some developments were made in the direction of low molecular weight analysis and these have already been summarized and discussed [30]. Focusing on the analysis of amino acids via MALDI-MS, some efforts were made including conventional MALDI matrices [29,31,32], ionic liquid matrices [33,34], carbon nanotubes [35–37], one basic MALDI matrix [38] and mesoporous silicate [39]. Nevertheless, difficulties concerning the analysis of amino acids like insufficient sensitivity still remain. Further as far as we know also the dependency of amino acid groups (acidic, aliphatic or other amino acids) on ionisation mode used has never been shown and discussed in detail.

Material enhanced laser desorption/ionisation-time of flight-mass spectrometry (MELDI-TOF-MS), based on the surface modification of silica gel with 4,4′-azo-dianiline or 3-amino-4-hydroxybenzoic acid, energy absorbing substances, was introduced in 2007 as a screening tool for the qualitative analysis of carbohydrates [30]. In this methodology, a thin layer of derivatised silica gel particles is positioned on the stainless steel target and analyte spotted on it. This layer provides the basis for analysis of low molecular weight analytes employing MALDI-MS.

The present effort is an important addition to tackle the challenges of analysing amino acids. As will be shown, MELDI-MS displays excellent sensitivity and performance for the analysis and screening of underivatised targets in positive and negative-ion mode. Finally, the analysis of biological samples like xylem saps highlights the potential of the method as a screening technology.

2. Experimental

2.1. Chemicals

All amino acids (glycine, alanine, serine, proline, valine, threonine, cysteine, hydroxyproline, leucine, asparagine, aspartic acid, glutamine, glutamic acid, methionine, histidine, phenyalanine, arginine, tyrosine, lysine, tryptophan, and cystine), methanol, acetone, and sodium chloride were obtained from Sigma–Aldrich (Sigma–Aldrich Chemie GmbH, Steinheim, Germany). Silica gel of pore size 1000 Å and particle size 35–70 μm was donated by Grace Vydac (Columbia, MD, USA). 4,4′-Azo-dianiline and 3-amino-4-hydroxybenzoic acid were purchased from Acros Organics (Geel, Belgium). Nitric acid (65%) was bought from Fluka (Buchs, Switzerland), 4,4′-diphenylmethane-diisocyanate, diethyl ether, pyridine, and acetonitrile from Merck AG (Darmstadt, Germany). All these chemical reagents were of analytical quality and used without further purification. Water purified by a Nano Pure-unit (Barnstead, Boston, MA, USA) was used.

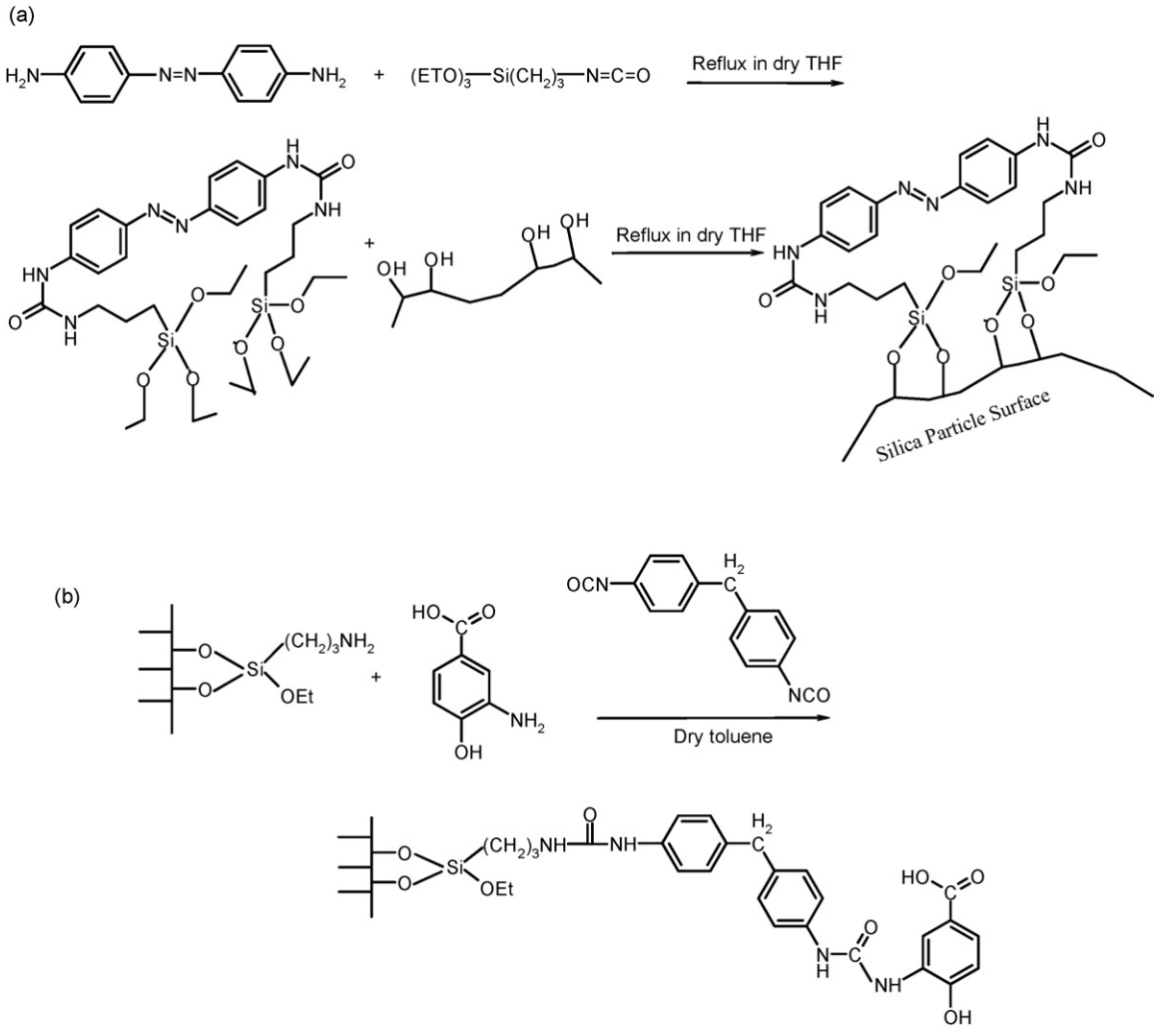

Fig. 1. Synthesis of 4,4′-azo-dianiline modified silica gel (a) and 3-amino-4-hydroxybenzoic acid modified silica gel (b).

2.2. Preparation of standards and samples

Individual amino acid standards were prepared at the concentration of 1 mg/mL in deionised water. For the mixture of amino acids, the concentration of each amino acid was 0.7 mg/100 mL. All these solutions were stored at 2–8 °C and diluted as required prior to measurements with purified water.

2.3. Xylem sap preparation

Twigs of from *Pinus cembra* L. (stone pine), *Betula pendula* Roth. (white birch), *Picea abies* (L.) Karst. (Norway spruce), and *Quercus robur* L. (oak) were harvested in April 2007 in the botanical garden of the University of Innsbruck, wrapped in plastic bags and transported to the laboratory. Side twigs were removed and up to 50 cm long sections of the main branches were prepared for sap extraction. Three replicates were collected, respectively.

Samples were enclosed in a pressure chamber (Super Chamber, PMS Instrument Company, Corvallis, OR, USA) with the basal end (about 5 cm) outside of the chamber's sealing lid. This end of the chamber was oriented downwards. Samples were exposed to pressures between 2 and 5 MPa for 15–60 min to enable extraction of at least 1 mL xylem sap, which was collected in small vials. After treatment, the xylem sap was immediately frozen and stored at −80 °C until analysis.

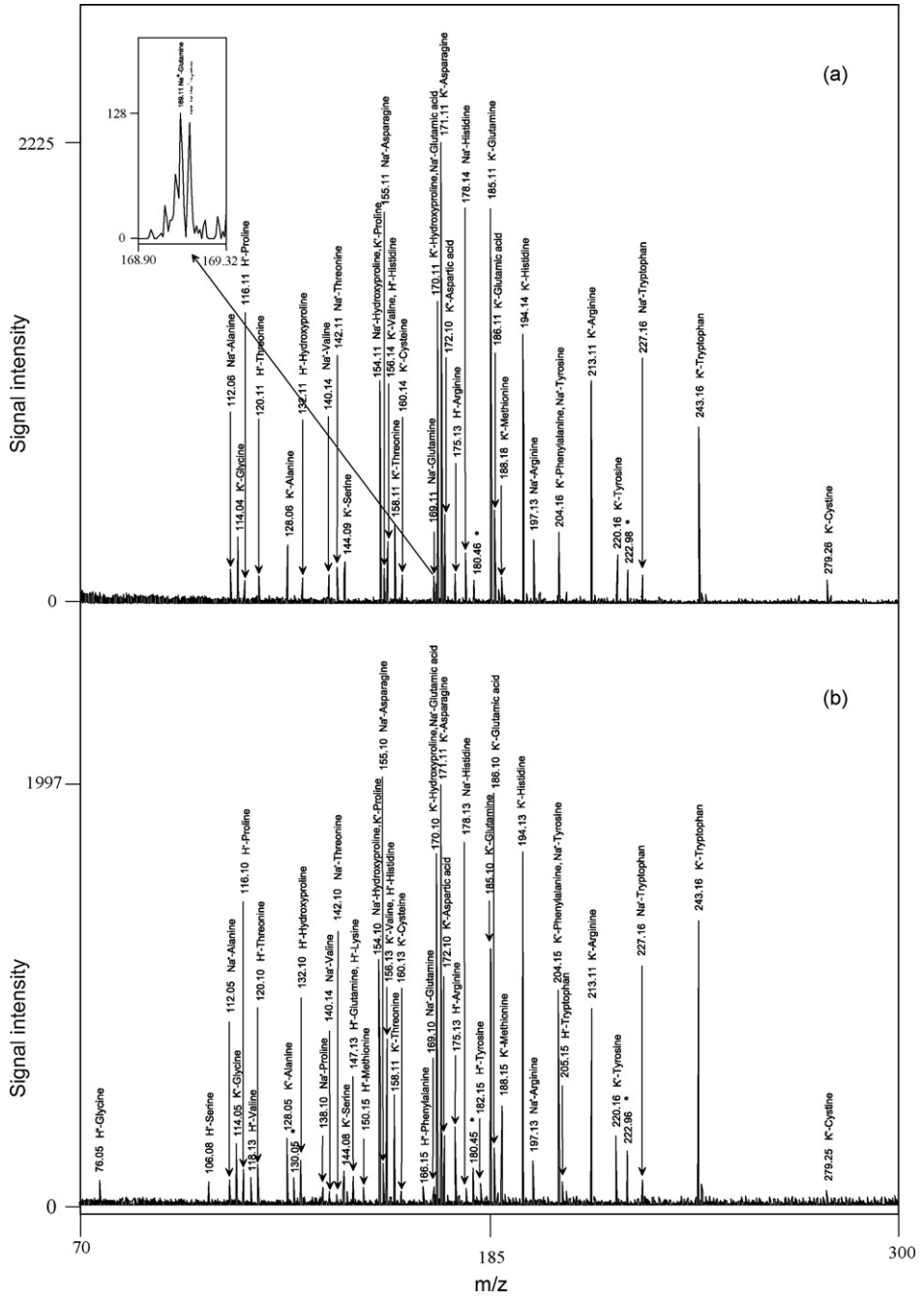

Fig. 2. MELDI-TOF-MS of amino acid mixture using 4,4′-azo-dianiline-silica (a) and 3-amino-4-hydroxybenzoic acid-silica (b) in positive-ion mode. Spectrum corresponds to 200 laser shots in each case. The absolute amount of each amino acid was 7 ng on the target in each case.

2.4. Instrumentation

Experiments were performed in positive-ion mode and negative-ion mode on a MALDI mass spectrometer (Ultraflex MALDI TOF/TOF, Bruker Daltonics, Bremen, Germany) employing stainless steel targets (MTP 384 target ground steel, Bruker Daltonics). Desorption was obtained using a 337 nm nitrogen laser and laser energy was adjusted as needed. Voltage impressed on the ion source one and two was 20.0 and 18.6 kV, respectively. Detection voltage was set at 1601 V. Flex Control (Version 2.0, Bruker Daltonics) was used for parameter control during recording and Flex Analysis (Version 2.0, Bruker Daltonics) for data evaluation. The number of laser shots used to obtain each spectrum was in the range from 200 to 500.

2.5. Energy absorbing surface materials for MELDI-MS

Two types of silica gel, one modified with 4,4′-azo-dianiline and the other one with 3-amino-4-hydroxybenzoic acid, were used as MELDI-MS surfaces (Fig. 1). Synthesis was performed according to the procedure described elsewhere [30].

2.6. On target sample preparation for MELDI-MS analysis

10 mg of modified silica gel was suspended separately in 1 mL of different solvent systems including methanol, acetone, acetonitrile, and acetonitrile/water (1:1). These were sonicated for 3 min. For MELDI-MS measurements, the suspension of modified material was applied as a thin layer on a stainless steel target and dried at room temperature. On this layer, 0.5–1 μL of sample solution was placed and dried under nitrogen gas.

3. Results and discussion

3.1. MELDI-MS for the analysis of amino acids

The 4,4′-azo-dianiline modified silica gel (ADA-silica) and the 3-amino-4-hydroxybenzoic acid modified silica gel (AHBA-silica) (Fig. 1) were both evaluated for the analysis of amino acids. Fig. 2 shows the mass spectrum of amino acid mixture analysed in the positive-ion mode using cited energy absorbing surface materials, i.e. ADA-silica and AHBA-silica, respectively. Silica gel particles employed were of the size 35–70 μm (1000 Å), as this type of silica delivered outstanding results concerning desorption and consequently provided the availability of carbohydrates for detection [30]. As noticeable from Fig. 2, sodium and potassium adducts of standards were detected alongside the protonated signals. Adduct formation of analytes in MALDI-MS [36] and in MELDI-MS [30] is not surprising, and it occurred despite the fact that samples were washed on the target by applying and removing several times 1 μL of pure water. Comparing the results from the two investigated energy absorbing surface materials, it is evident that by using AHBA-silica more standards deliver their protonated $[M+H]^+$, sodium $[M+Na]^+$ and potassium $[M+K]^+$ adducts, i.e. the usual set of quasi-molecular ions. This means that more analytes are detected in the protonated form besides their sodium and potassium adducts.

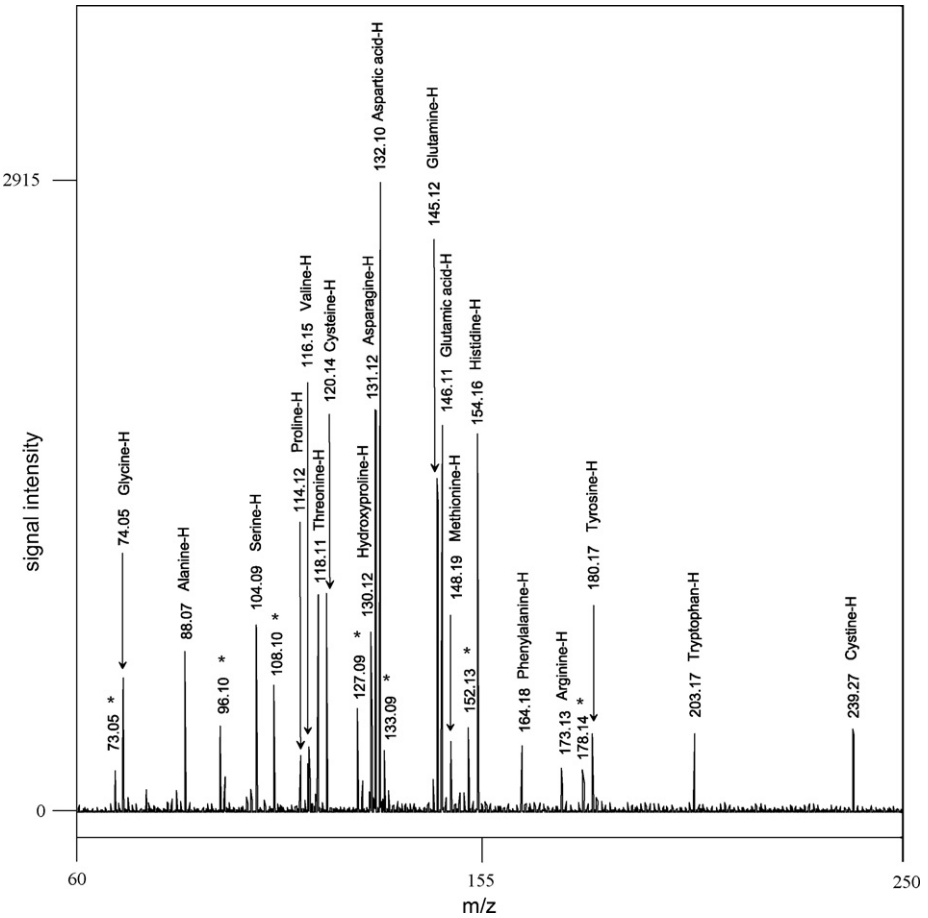

Fig. 3. MELDI-TOF-MS of amino acid mixture using 4,4′-azo-dianiline-silica in negative-ion mode. Spectrum corresponds to 200 laser shots. The absolute amount of each amino acid was 7 ng on the target.

This can be ascribed to the nature of the energy absorbing surface material, as AHBA-silica retains free acidic functions and can, therefore, donate protons. In the case of AHBA, acidic amino acids like aspartic acid and glutamic acid as well as some other amino acids like alanine, cysteine, asparagine and cystine (dimeric form of cysteine) do not deliver protonated ions. In the case of ADA, no acidic function is present on the surface, which is why nearly all analytes are detected only as sodium and potassium adducts. Nevertheless there are also some exceptions, such as the basic amino acids histidine and arginine in addition to proline, hydroxyproline and threonine. The absence of protonated signals did not influence negatively, as presence of sodium and potassium adduct signals clearly allowed signals assignment. But a negative aspect is the determination of amino acids showing nearly the same mass, like glutamine (146.15 Da) and lysine (146.19 Da) or leucine (131.17 Da), isoleucine (131.17 Da) and hydroxyproline (131.13 Da). Only by employing a high resolution mass spectrometer these analytes can be clearly detected as single substances.

For both energy absorbing surface materials, exactly the same behaviour concerning adduct formation of analytes can be found, i.e. all analytes were detected as potassium adducts. They were detected also as sodium adducts except for the glycine, serine, cysteine, aspartic acid, methionine, phenylalanine and cystine. With the exception of the influence of energy absorbing surface materials on the analysis, no significant differences between them could be found for the investigation of amino acids. In fact, signal intensities and signal to noise ratios were approximately the same. Besides the identification of amino acids in the mixture, two less intense signals (at m/z 180.4 and 222.9) were also found. The nature of these is still not resolved.

Table 1
Results from MELDI-TOF-MS measurements of amino acids mixture employing 4,4′-azo-dianiline-silica in positive-ion mode and negative-ion mode.

Amino acids (mixture)	Theoretical masses	Measured masses			
		Positive-ion mode		Negative-ion mode	
		m/z	Cations	m/z	Anions
Glycine	75.07	114.04	$[M+K]^+$	74.05	$[M-H]^-$
Alanine	89.09	112.06 128.06	$[M+Na]^+$ $[M+K]^+$	88.07	$[M-H]^-$
Serine	105.09	144.09	$[M+K]^+$	104.09	$[M-H]^-$
Proline	115.13	116.11 154.11	$[M+H]^+$ $[M+K]^+$	114.12	$[M-H]^-$
Valine	117.15	140.14 156.14	$[M+Na]^+$ $[M+K]^+$	116.15	$[M-H]^-$
Threonine	119.12	120.11 142.11 158.11	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$	118.11	$[M-H]^-$
Cysteine	121.16	160.14	$[M+K]^+$	120.14	$[M-H]^-$
Hydroxyproline	131.13	132.11 154.11 170.11	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$	130.12	$[M-H]^-$
Leucine	131.17	Not detected	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$	Not detected	
Asparagine	132.12	155.11 171.11	$[M+Na]^+$ $[M+K]^+$	131.12	$[M-H]^-$
Aspartic acid	133.10	172.10	$[M+K]^+$	132.10	$[M-H]^-$
Glutamine	146.15	169.11 185.11	$[M+Na]^+$ $[M+K]^+$	145.12	$[M-H]^-$
Lysine	146.19	169.15	$[M+Na]^+$	Not detected	
Glutamic acid	147.13	170.11 186.11	$[M+Na]^+$ $[M+K]^+$	146.11	$[M-H]^-$
Methionine	149.21	188.18	$[M+K]^+$	148.19	$[M-H]^-$
Histidine	155.16	156.14 178.14 194.14	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$	154.16	$[M-H]^-$
Phenylalanine	165.19	204.16	$[M+K]^+$	164.18	$[M-H]^-$
Arginine	174.20	175.13 197.13 213.11	$[M+H]^+$ $[M+Na]^+$ $[M+K]^+$	173.13	$[M-H]^-$
Tyrosine	181.19	204.16 220.16	$[M+Na]^+$ $[M+K]^+$	180.17	$[M-H]^-$
Tryptophan	204.23	227.16 243.16	$[M+Na]^+$ $[M+K]^+$	203.17	$[M-H]^-$
Cystine	240.32	279.26	$[M+K]^+$	239.27	$[M-H]^-$

When working in the negative-ion mode, almost all investigated amino acids could be detected as their anions in the case of ADA-silica (Fig. 3). With AHBA-silica, analysis of amino acids was very difficult, as noise dominated the spectrum and the signal intensity of analytes was very low (data not shown). The reason for this is again related to the surface chemistry of the employed MELDI materials, as the difference in proton affinities (PA) between the energy absorbing surface materials and analytes plays a crucial role in MELDI-MS. This phenomenon relates to secondary ionisation events, where charge transfer, proton transfer, metal ion transfer, etc. takes place from matrix to analyte molecules in the expanding plume and vice versa [40].

A strong capability of AHBA-silica to donate protons influences negatively the formation of anions. By contrast, for ADA-silica, the high proton affinity of the energy absorbing surface material enhances the detection of anions. As a result, only when working with ADA-silica, positive- and negative-ion mode can be employed. Two further observations can be made: In the positive-ion mode, qualitative analysis is more reliable as analytes are detected at least by two signals (Fig. 2a). In the negative-ion mode, the resulting spectra are less complex with higher signal intensities (Figs. 3 and 5). Therefore, positive- and negative-ion modes have to be used for correct qualitative analysis and data interpretation in order to obtain highly reliable results (Table 1).

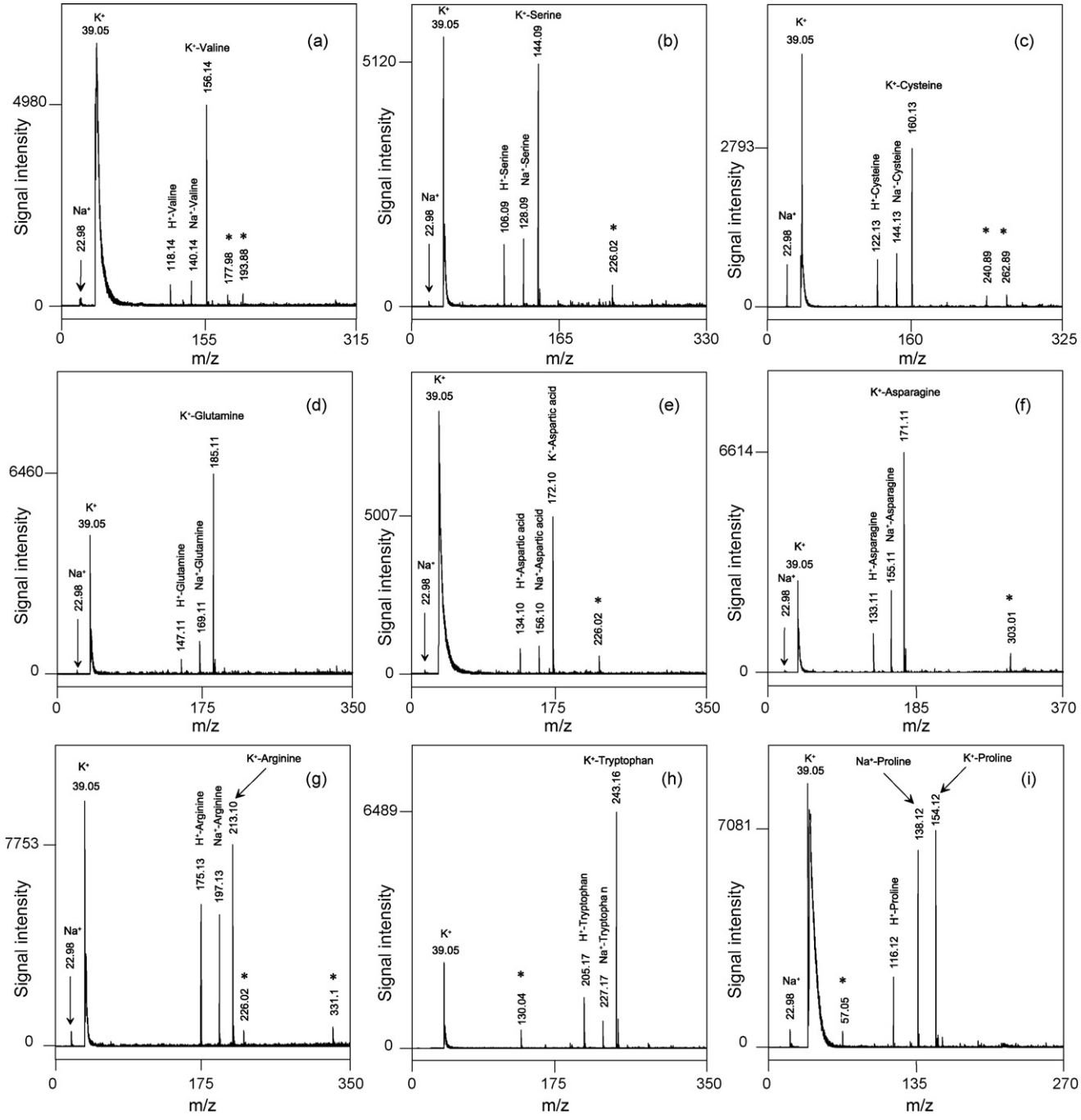

Fig. 4. MELDI-TOF-MS of single amino acids using 4,4′-azo-dianiline-silica in positive-ion mode. (a) Valine, (b) serine, (c) cysteine, (d) glutamine, (e) aspartic acid, (f) asparagines, (g) arginine, (h) tryptophan and (i) proline. Each spectrum corresponds to 400 shots. The absolute amount of analytes was 0.5 μg on the target in each case.

An interesting finding can be observed when measuring amino acid as single standards. Fig. 4 depicts the analysis of single amino acids in the positive-ion mode using ADA-silica as energy absorbing surface material for their desorption. For every amino acid three signals were detected although only rarely protonated signals were gained within the mixture. A few unidentified low intensity signals were also found e.g. a signal at m/z 226 is present in serine, aspartic acid and arginine. In the negative mode, signal intensities were always higher than in the positive ionisation mode with excellent signal to noise ratio (Fig. 5).

Generally, amino acids are classified into several groups depending on the nature of their side chain, i.e. aliphatic, aromatic, acidic, basic, acid amides, imines and sulphur containing amino acids. Alterman et al. [29] clearly demonstrated that signal intensity is dependent primarily on nature of amino, as he compared the signal intensity of analytes in the range of 300–20 μM. It is evident that not every group will be ionised with the same efficiency as others. In fact, acidic amino acids deprotonate easily and should therefore deliver high signals when working in the negative-ion mode. On the other hand, basic amino acids protonate easily and

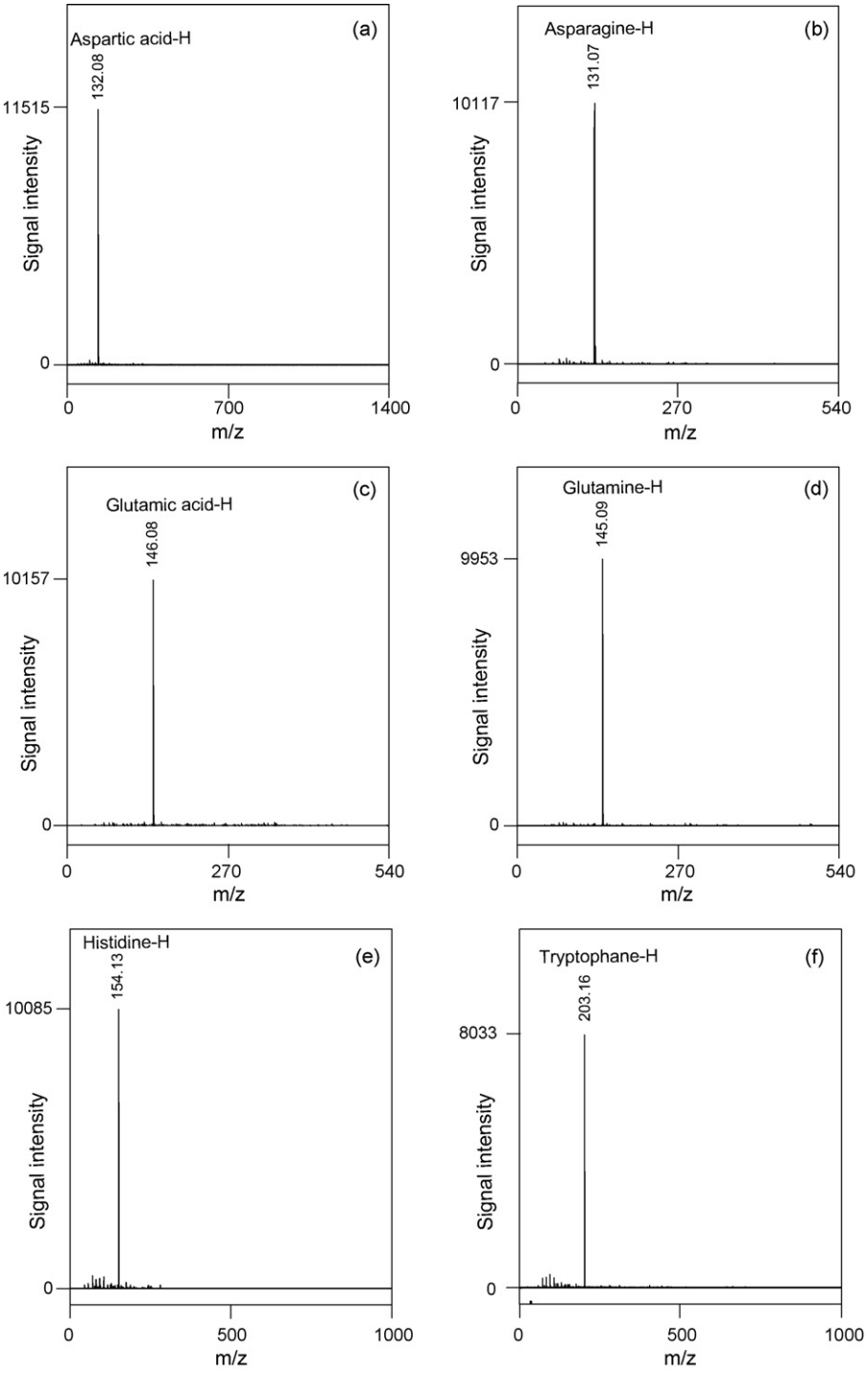

Fig. 5. MELDI-TOF-MS of single amino acids using 4,4′-azo-dianiline-silica in negative-ion mode. (a) Aspartic acid, (b) asparagines, (c) glutamic acid, (d) glutamine, (e) histidine and (f) tryptophan. Each spectrum corresponds to 400 shots. The absolute amount of analytes was 0.5 μg on the target in each case.

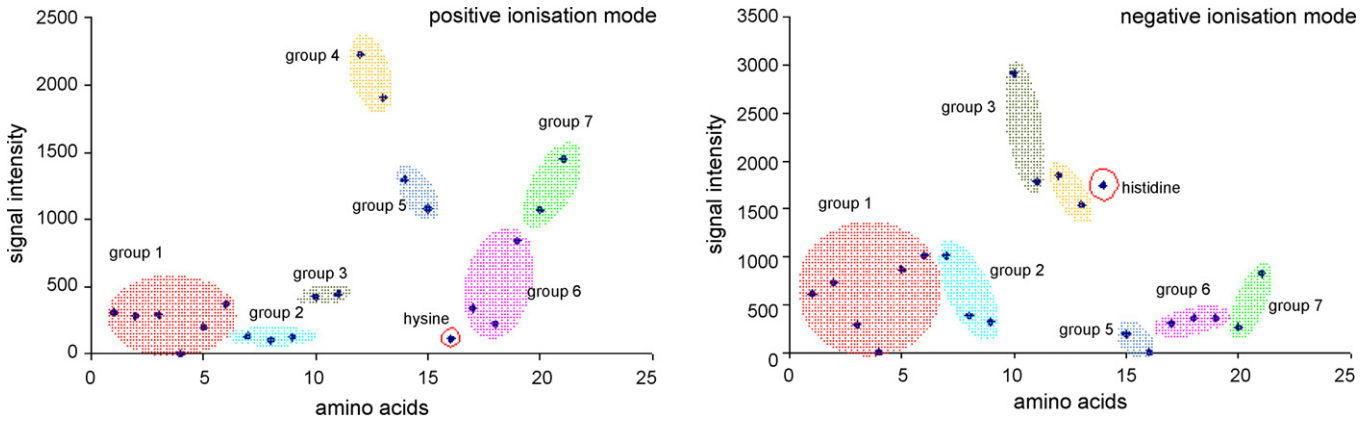

Fig. 6. Signal intensities of single amino acids working in different ion modes. Red circles mark outliers, group 1: aliphatic amino acids (1. glycine, 2. alanine, 3. valine, 4. leucine, 5. serine, 6. threonine), group 2: sulphur/thiol containing amino acids (7. cysteine, 8. cystine, 9. methionine), group 3: acidic amino acids (10. aspartic acid, 11. glutamic acid), group 4: amides (12. asparagine, 13. glutamine), group 5: basic amino acids (14. histidine, 15. arginine, 16. lysine), group 6: aromatic amino acids (17. phenylalanine, 18. tyrosine, 19. tryptophan) and group 7: imines (20. proline, 21. hydroxyproline).

should therefore be detected at high signal intensities when working in the positive-ion mode. The question is: how are other amino acid groups behaving in respect to the ionisation mode chosen? Fig. 6 shows the connection between signal intensity and nature of analyte (results based on Figs. 2a and 3). The behaviour of acidic (group 3) and basic (group 5) amino acids exactly follows the rules as postulated. Among the basic amino acids histidine was an exceptional case and the reason for its strong intensity in negative-ion mode is not fully clear. Measurements of amides (group 4) showed that they deliver almost similarly high signal intensities, indepen-

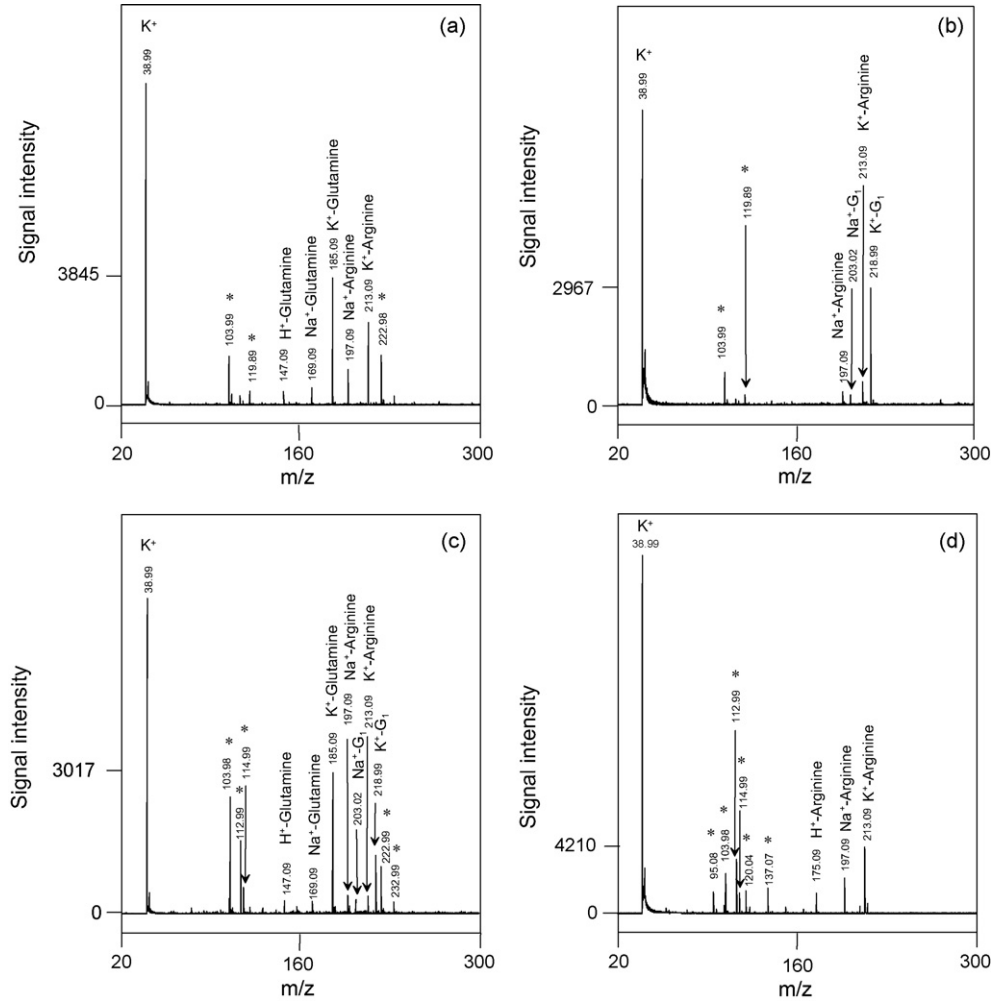

Fig. 7. MELDI-TOF-MS of different xylem sap samples using 4,4′-azo-dianiline-silica in positive-ion mode. (a) *Pinus cembra* L., (b) *Betula pendula* Roth., (c) *Picea abies* L., and (d) *Quercus robur* L. G_1 = hexose; (*) not identified. Each spectrum corresponds to 500 laser shots.

Table 2
Results from MELDI-TOF-MS screening of xylem sap samples from different plant species employing 4,4′-azo-dianiline-silica in positive-ion mode and negative-ion mode.

Xylem sap samples	Cations		Anions	
	m/z	Compounds detected	*m/z*	Compounds detected
Pinus cembra L.	38.99	K^+	132.99	[Glutamine−H]$^-$
	103.99		145.12	
	119.89	[Glutamine+H]$^+$	153.07	
	147.09	[Glutamine+Na]$^+$	167.07	
	169.09	[Glutamine+K]$^+$	193.07	
	185.09	[Arginine+Na]$^+$		
	197.09	[Arginine+K]$^+$		
	213.09			
	222.98			
Betula pendula Roth.	38.99	K^+	148.99	
	103.99		164.99	
	119.89	[Arginine+Na]$^+$ [G_1+Na]$^+$	180.89	
	197.09	[Arginine+K]$^+$		
	203.02	[G_1+K]$^+$		
	213.09			
	218.99			
Picea abies (L.) Karst.	38.99	K^+	132.09	[Aspartic acid−H]$^-$
	103.98		145.12	[Glutamine−H]$^-$
	112.99	[Glutamine+H]$^+$	153.09	
	114.99	[Glutamine+Na]$^+$	164.99	
	147.09	[Glutamine+K]$^+$	180.99	
	169.09	[Arginine+Na]$^+$	228.89	
	185.09	[G_1+Na]$^+$	244.89	
	197.09	[Arginine+K]$^+$		
	203.02	[G_1+Na]$^+$		
	213.09			
	218.99			
	222.99			
	232.99			
Quercus robur L.	38.99	K^+	164.99	
	95.08		183.89	
	103.98	[Arginine+H]$^+$	228.89	
	112.99	[Arginine+Na]$^+$ [Arginine+K]$^+$		
	114.99			
	120.04			
	137.07			
	175.09			
	197.09			
	213.09			

dent from the selected ionisation mode. Only acidic amino acids surpassed these values in the negative-ion mode (Fig. 6). In the case of sulphur/thiol (group 2) containing amino acids, the lowest values in comparison to all others were gained in the positive-ion mode. Interestingly, even group 3 (acidic amino acids) delivered higher intensities compared with group 2. Also in the negative-ion mode the behaviour of group 2 did not differ very much. In this case, only basic amino acids (group 5) delivered lower val-

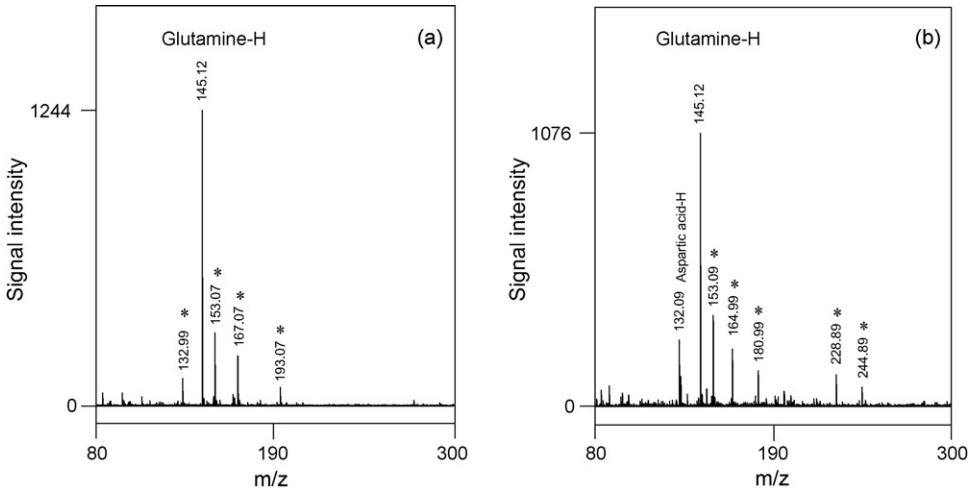

Fig. 8. MELDI-TOF-MS of different xylem sap samples using 4,4′-azo-dianiline-silica in negative-ion mode. (a) *Pinus cembra* L., (b) *Picea abies* L. (*) not identified. Each spectrum corresponds to 200 laser shots.

ues. Amino acids with an aromatic side chain behaved similarly (group 6). Aliphatic amino acids are defined by the presence of a methyl, an ethyl, or an isopropyl group and sometimes also by the presence of an additional hydroxyl group in the side chain. In positive- and in negative-ion mode, this group (group 1) delivered rather low signal intensities, i.e. a maximum of one third of the absolute value in comparison to the highest signal values detected (Fig. 6).

Finally, the detection limit of MELDI-MS when working with ADA-silica was determined in positive-ion mode at different concentrations using amino acid standards (arginine, histidine and glutamine) in the range of 0.25 μg/μL (1.2 nmol/μL) to 0.73 pg/μL (5 fmol/μL). Results have shown that amino acids can be easily detected down to a concentration of 2.10 pg/μL (10 fmol/μL) and signal to noise at this point was 10:1.

3.2. Screening of xylem sap samples

Xylem saps from *Picea cembra* L. (stone pine), *Betula pendula* Roth. (white birch), *Picea abies* (L.) Karst. (Norway spruce), and *Quercus robur* L. (oak) were analysed via MELDI-MS using ADA-silica. In the *Picea cembra* sap, all three adducts of glutamine, $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$, were detected alongside the sodium and potassium adducts of arginine, $[M+Na]^+$ and $[M+K]^+$ (Fig. 7a). Glutamine is known to be an important transporter for nitrogen [41,42]. In regard to *Betula pendula*, there was a clear indication of the presence of sodium and potassium adducts of arginine and sodium and potassium adducts of a hexose sugar represented as G_1 (Fig. 7b). In *Picea abies*, glutamine was detected in the form of $[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$ and arginine as its $[M+Na]^+$ and $[M+K]^+$ beside $[M+Na]^+$ and $[M+K]^+$ of a hexose (Fig. 7c). In the *Quercus robur* xylem sap only arginine could be detected in the form of $[M+H^+]$, $[M+Na^+]$ and $[M+K^+]$ (Fig. 7d). Besides the identification of analytes in all four samples, some other signals were detected. The nature of these signals is still not resolved and shows the limit of the introduced method. Nevertheless, non-identified signals can also be used for differential analysis. This means that differences and similarities between samples can be found on the basis of clearly identified analytes and on the basis of unknown detected signals. For example, signal at m/z 103.9 is present in all spectra while a signal at m/z 114.9 is present only in saps from *Picea abies* and *Quercus robur* (Table 2).

Finally, Fig. 8 shows the analysis of xylem saps from *Picea cembra* and *Picea abies* in the negative ionisation mode. In *Picea cembra* (Fig. 8a), glutamine is detected as its deprotonated form while for *Picea abies* L. (Fig. 8b), two amino acids, glutamine and aspartic acid, were detected. All other peaks marked with asterisks in both spectra are not yet known.

4. Conclusions

This study presents material enhanced laser desorption/ionisation mass spectrometry (MELDI-MS) as a fast and reliable screening technology for the qualitative analysis of amino acids. The most important and interesting data was available for the comparison of amino acids in positive- and negative-ion mode. 4,4′-Azo-dianiline derivatised MELDI material delivered excellent signal to noise ratio and signal intensity without background noise, suggesting a great potential of the technique in the field of MALDI-MS. Limit of detection was determined, delivering values of 2.10 pg. The analysis of real samples such as xylem saps from different trees proves the viability of the method as a fast and reliable screening technology. The excellent performance of presented MELDI system in positive- and negative-ion mode is unique in the field of MALDI-MS for the analysis of amino acids.

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References

[1] T. Saga, D.N. Heiger, Anal. Chem. 72 (2000) 1236.
[2] M. Eizo, New Food Ind. 38 (1996) 59.
[3] F. Mendel, G.R. Micheal, B.L. David, Front. Gastr. Res. 14 (1988) 79.
[4] A.K. Misra, S. Pachaures, Acta Chromatogr. 12 (2002) 189.
[5] T. Hodisan, M. Culea, C. Cimpoiu, A. Cot, J. Pharm. Biomed. Anal. 18 (1998) 319.
[6] W. Chu, M. Shinimiya, K.Y. Kamitori, S. Kamitori, R.G. Carlson, R.F. Weaver, F. Takusagawa, J. Am. Chem. Soc. 116 (1994) 7971.
[7] A.M. Altschul, L.E. Castillon, US Pat. Appl. Publ. US 2582949 19520122 (1952).
[8] R.L. Heinrikson, S.C. Meredith, Anal. Biochem. 136 (1984) 65.
[9] P. Fuerst, L. Pollack, T.A. Graser, H. Godel, P. Stehle, J. Chromatogr. 499 (1990) 557.
[10] V. Fierabracci, P. Masiello, M. Novelli, E. Bergamini, J. Chromatogr. 570 (1991) 285.
[11] R. Schuster, J. Chromatogr. 431 (1988) 271.
[12] M. Griffin, J. Leah, N. Mould, G. Compton, J. Chromatogr. 431 (1988) 285.
[13] G.J. Schmidt, D.C. Olson, W. Slavin, J. Liq. Chrom. 2 (1979 1031).
[14] B.K. Singh, B. Tecle, D.L. Shaner, J. Liq. Chrom. 17 (1994) 4469.
[15] B.V. Charlwood, A.E. Bell, J. Chromatogr. 135 (1977) 377.
[16] E. Zahou, H. Joernvall, T. Bergman, Anal. Biochem. 281 (2000) 115.
[17] A. Taga, S. Honda, J. Chromatogr. A 742 (1996) 243.
[18] J.T. Smith, Electrophoresis 20 (1999) 3078.
[19] T. Soga, G.A. Ross, J. Chromatogr. A 837 (1999) 231.
[20] R.W. Charless, A.A. Mark, J. Chromatogr. A 837 (1999) 231.
[21] C.W. Oh, T.J. Mabry, K.R. Kim, J.H. Kim, J. Chromatogr. Sci. 33 (1995) 399.
[22] I.M. Perl, Z.F. Katona, Chromatogr. Sup. 51 (2000) S-228.
[23] M. Zampolli, D. Meunier, R. Sternberg, F. Raulin, C. Szopa, M.C. Pietrogrande, F. Dondi, Chirality 18 (2006) 279.
[24] R.G.J. van Leuken, A.L.L. Duchateau, G.T.C. Kwakkenbos, J. Pharm. Biomed. Anal. 13 (1995) 1459.
[25] T. Kurahashi, A. Miyazaki, S. Suwan, M. Isobe, J. Am. Chem. Soc. 123 (2001) 9268.
[26] J. Kim, S. Park, N.Y. Tretyakova, C.R. Wagner, Mol. Pharm. 2 (2005) 233.
[27] D. Fekkes, J. Chromatogr. B 682 (1996) 3.
[28] K. Petritis, P. Chaimbault, C. Elfakir, M. Dreux, J. Chromatogr. A 896 (2000) 253.
[29] M.A. Alterman, N.V. Gogichayeva, B.A. Kornilayev, Anal. Biochem. 335 (2004) 184.
[30] M.A. Hashir, G. Stecher, R. Bakry, S. Kasemsook, B. Blassnig, I. Feuerstein, G. Abel, M. Popp, O. Bobleter, G.K. Bonn, Rapid Commun. Mass Spectrom. 21 (2007) 2759.
[31] J.E. Dally, J. Gorniak, R. Bowie, C.M. Bentzley, Anal. Chem. 75 (2003) 5053.
[32] N.V. Gogichayeva, T. Williams, M.A. Alterman, J. Am. Soc. Mass Spectrom. 18 (2007) 279.
[33] M. Zabet-Moghaddam, E. Heinzle, A. Tholey, Rapid Commun. Mass Spectrom. 18 (2004) 141.
[34] M. Zabet-Moghaddam, R. Krueger, E. Heinzle, A. Tholey, J. Mass Spectrom. 39 (2004) 1494.
[35] J. Zhang, H.Y. Wang, Y.L. Guo, Chin. J. Chem. 23 (2005) 185.
[36] C. Pan, S. Xu, L. Hu, X. Su, J. Ou, H. Zou, Z. Guo, Y. Zhang, B. Guo, J. Am. Soc. Mass Spectrom. 16 (2005) 883.
[37] C. Wang, J. Li, S. Yao, Y. Guo, X. Xia, Anal. Chim. Acta 604 (2007) 158.
[38] R. Shroff, A. Muck, A. Svatos, Rapid Commun. Mass Specrom. 21 (2007) 3295.
[39] C.S. Lee, J.H. Lee, K.K. Kang, H.M. Song, I.H. Kim, H.K. Rhee, B.G. Kim, Biotech. Bioprocess. Eng. 12 (2007) 174.
[40] R. Knochenmuss, R. Zenobi, Chem. Rev. 103 (2003) 441.
[41] R. Lösch, Wasserhaushalt der Pflanzen, Quelle & Meyer, Wiebelsheim/Germany, 2003, p. 595.
[42] A.D. Peuke, W.D. Jeschke, J. Exp. Bot. 44 (1993) 1167.