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2B or not 2B, that is the question: further investigations into the use of pencil as a matrix for matrix-assisted laser desorption/ionisation

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The effective use of pencil as a matrix for matrix-assisted laser desorption/ionisation (MALDI) for the study of actinides has previously been demonstrated (Black *et al.*, *Rapid Commun. Mass Spectrom.* 2006; 20: 1053). Here, the scope of the types of molecules amenable to analysis by this method has been extended, establishing that ~90% of a library containing 50 diverse small molecules can be successfully analysed by this technique. Further, the role played by the bulk materials present in the different pencil leads has been investigated and a simple one-step deposition of matrix and calibration materials has been achieved through the fabrication of different calibration pencils (Cali-Pens).

The use of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOFMS) has become widespread since the pioneering work of Tanaka *et al.*[1] and Karas and Hillenkamp.[2] Subsequently, a wide range of matrices, mainly organic in nature, has been utilised. Ions produced from these matrices generally appear in the low-mass region, below *m/z* 500, and so hinder or prohibit the acquisition of useful data in this mass region. Cohen and Gusev discussed a number of alternative methods to improve the analysis of low molecular weight compounds by MALDI.[3] These included studies using conventional crystalline matrices to analyse small peptides,[4] carbohydrates,[5] and polymers.[6] As well as producing interference ions, crystalline matrices are also known to have a number of disadvantages for the analysis of low molecular weight compounds in that signal reproducibility may be poor from shot to shot and between samples, and the need to search for a 'sweet-spot' can sometimes be time-consuming.[3] High molecular weight,[7] liquid,[8] inorganic and two-phase matrices[1] have all been utilised to overcome some of these problems. More recently, the use of ionic liquid matrices has become widespread[9] and the conditions required for the effective use of the matrix suppression effect (MSE) have been investigated.[10] The use of graphite as a matrix[11–13] and the utilisation of graphite target plates, without the addition of a standard crystalline matrix,[14] was discussed in our earlier publication,[15] where graphite powder and graphite rod were investigated as possible matrices for the analysis of actinides. Difficulties in handling and deposition of the graphite were highlighted and a simplified method utilising pencil lead (PL) as a new MALDI matrix was developed in this laboratory to address these problems. One of the advantages of using pencil is that it offers a far simpler method of applying a matrix to the MALDI plate. The pencil is simply scribbled onto the target spot producing a hydrophobic surface that allows a sample droplet to dry to a concentrated spot.[15] As well as proving to be an excellent matrix for actinide analysis, some promise was shown for a few small organics, peptides and small polymers studied. To investigate this further, a diverse library of small molecules has been analysed to determine the breadth of application of PL-MALDI and the results are presented here. Application of this approach is further expanded for the analysis of lanthanides, since this matrix proved to be so successful for the detection of the actinides.

Pencils come in many different grades, according to the ratio of clay to graphite, as first discovered by Nicholas Jacques Conté in 1795.[16] By baking the graphite with clay in specific ratios the hardness of the pencil, where H = Hardness and B = Blackness, can be controlled instead of simply relying on the purity of graphite. The higher the ratio of clay to graphite, the harder the pencil—these are 'H' pencils that leave a fainter mark. The higher the ratio of graphite to clay, the softer the pencil—these are 'B' pencils that leave a blacker mark. Pencil hardness ranges from 9H through to 9B (in the USA, a numbered system exists). It has previously been discovered that it was easier to obtain increased ion signal intensity with pencils of a higher black rating (higher B numbers).[15] However, pencils do not only contain clay and graphite. In order to give a smooth writing tool, waxes and oils are impregnated into the pencil leads during manufacture; these have been termed 'glide agents'. Different makes of pencil contain a variety of different additives, i.e. various waxes and oils, dependent on the individual manufacturing procedures. The role played by these materials, i.e. the clays and glide agents,

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in respect to their use as MALDI matrices, has also been investigated.

Since the use of pencil lead has proved to be successful for MALDI, a simple calibration pencil (Cali-Pen) has been fabricated where individual pencil leads are impregnated with known reference materials. By selecting the appropriate pencil a simple and easy calibration can be obtained to mass calibrate, verify and check instrument performance.

EXPERIMENTAL

All standard MALDI matrices except the pencils were purchased from Sigma-Aldrich (Gillingham, UK). Methanol, dichloromethane, acetone and formic acid were purchased from Fisher Scientific (Loughborough, UK). The pencils used were a mix of different sets from various manufacturers and suppliers including Staedtler (Nuernberg, Germany), Corporate, Niceday, Dixon® and Woolworths.

2,5-Dihydroxybenzoic acid (DHB) was prepared as a 3 mg/mL solution in 50:50 acetonitrile/water (0.1% formic acid) and α-cyano-4-hydroxycinnamic acid (CHCA) matrices were prepared as (i) a saturated solution in 50:50 acetonitrile/water (0.1% formic acid) and (ii) a saturated acetone solution. All other samples were acquired from sources in the School of Chemistry, University of Southampton, and were prepared as approximately 10 μg/mL solutions in methanol.

MALDI-TOF mass spectra were acquired using a Micromass (Manchester, UK) TofSpec2E MALDI time-of-flight (TOF) mass spectrometer with a 337 nm nitrogen laser. The instrument was operated in positive ion reflectron mode at all times. The laser power was standardised to 50% coarse and 50% fine in order to compare spectra. For the LDI (laser desorption/ionisation) work the maximum laser power (100% coarse, 100% fine) was used to determine whether self-matrix properties were observed. At least 20 laser shots were collected for each spectrum obtained. All spectra were processed using one Savitsky-Golay smoothing with a smooth window of 1 and a background subtraction where the polynomial order was 25, below curve set to 40% and the tolerance was 0.01. The sample (1 μL) was pipetted on top of a thin layer of pencil, or mixed with the organic matrix solutions on the MALDI target plate, and left to dry in air.

Gas chromatography (GC) mass spectra were acquired using a Thermo Trace GC/MS system (Thermo Electron; San José, CA, USA). The 70 eV electron ionisation (EI) data were recorded with ion source temperature of 200°C and trap current of 150 μA. A ZB5-MS column, 30 m × 0.25 mm, 0.25 μm film thickness (Phenomenex, Torrance, CA, USA), was used for all analyses. The injector temperature was 220°C and the oven temperature was set at 40°C for 4 min rising to 160°C at 10°C/min, then at 2°C/min to 220°C, and finally at 10°C/min up to 320°C, where it was held for 5 min. Helium carrier gas was used at a constant flow rate of 1 mL/min. A 5 mm piece of pencil lead was removed from each pencil and placed in a vial with 1 mL of dichloromethane and left for 24 h. A 1 μL volume of the supernatant was injected into the gas chromatograph using an AS800 autosampler.

High-resolution electrospray ionisation (ESI) mass spectra were acquired using a Bruker Daltonics (Billerica, MA, USA) Apex III Fourier transform ion cyclotron resonance (FTICR) mass spectrometer, equipped with a 4.7 Tesla actively shielded superconducting magnet and an Infinity cylindrical analyser cell. The samples were introduced using an Apollo off-axis ESI source at a flow rate of 3 μL/min.

RESULTS AND DISCUSSION

From the library of diverse small molecules, containing tripeptides, steroids, drugs, herbicides, dyes, polyethylene glycols (PEGs), etc., 88% of the compounds were successfully analysed by PL-MALDI-TOFMS (see Table 1). To be classified as a successful analysis, ions enabling the relative molecular mass of each molecule to be identified must be observed at a signal-to-noise (S/N) ratio of at least 5:1. Whilst ~25% of the successfully detected molecules exhibit some self-matrix properties (indicating that they could be ionised without the presence of a matrix, i.e. laser desorption/ionisation, LDI), in all cases the ionisation was significantly enhanced in the presence of pencil matrix. By using the same laser power and number of laser shots for each analysis no signal was observed under LDI conditions for any compound (see Fig. 1(ii)). Only on increasing the laser power to 100% coarse, 100% fine, was any LDI signal recorded (see Fig. 1(iii) and Table 1). For the latter the S/N ratio was 7:1 compared with 70:1 for the PL-MALDI recorded at the lower laser power (see Fig. 1(i)).

Not only does application of the matrix to the MALDI plate simplify the sample preparation, it also produces spectra with little or no matrix ion interference (see Figs. 2(a), 2(b) and 2(c). It can be seen in Fig. 2(a), the tripeptide, Ala-Leu-Gly (relative molecular mass 259), and in Fig. 2(b), the steroid, dexamethasone (relative molecular mass 392), that the analysis using classical organic matrices is compromised by the presence of matrix and associated cluster ions. Identification of the protonated molecule, *m/z* 260, or the cationised Ala-Leu-Gly molecule, *m/z* 282 ($[M+Na]^+$) and *m/z* 298 ($[M+K]^+$), is difficult due to the presence of other dominant ions including, e.g., *m/z* 379 ($[2CHCA+H]^+$), and *m/z* 441 and 443, believed to be $[2CHCA+Cu]^+$. The latter is a common adduct always observed when using CHCA on the TofSpec sample plates. (Note also the ions at *m/z* 322 and 324 in Fig. 2(a), $[M+Cu]^+$ for Ala-Leu-Gly.) The result is similar for dexamethasone where the sodiated molecule, *m/z* 415, is of low abundance in the CHCA mass spectrum. This identification process is further complicated for any compound where the relative molecular mass is unknown.

For PL-MALDI the cationised molecules are observed at significantly improved S/N ratio compared with the classical organic matrices and the resultant mass spectra are mainly devoid of interfering ions (see Fig. 2(c), (iii)). Here, for erythromycin ethyl succinate, the potassiated molecule, *m/z* 900, is the base peak; identification of this ion is aided by the presence of the protonated and sodiated molecules at *m/z* 862 and 884, respectively. Two other ions are prominent in this spectrum, *m/z* 740 thought to be a fragment ion due to deglycosylation of the potassiated molecule (*m/z* 900), and *m/z* 772, the latter interpreted as $[M+K]^+$ for erythromycin, produced as a degradation product from erythromycin ethyl

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Table 1. PL-MALDI results for diverse small molecule library (✓ = successful analysis where signal-to-noise (S/N) ratio is >5:1, × = no ions observed)

Compound	PL	Self Matrix	Compound	PL	Self Matrix
α-Cyclodextrin	✓		Erythromycin ethyl succinate	Fragment	
4-Hydroxy Tamoxifen	✓	weak	Erythromycin stearate	✓	
Alachlor	×		Haloperidol	✓	
Ala-Leu-Gly	✓		Lignocaine	✓	weak
Ala-Pro-Gly	✓		Mebendazole	✓	weak
Atropine	✓		Methoxamine	✓	
Bensulfuron methyl	✓		Methyl Red	✓	weak
Betamethasone	✓		Norethindrone	✓	
Caffeine	✓		Oxibendazole	✓	weak
Captopril	✓	weak	Oxybutynin	✓	
Carbamazepine	✓	weak	PEG400	✓	
Carbaryl	✓		PEG1000	✓	
Carbendazim	✓		Pimozide	✓	
Cefuroxime	×		Pyrazosulphuron ethyl	×	
Cefuroxime Axetil	✓		Quinine	✓	weak
Chlorimuron ethyl	✓		Ranitidine	✓	weak
Chlorsulphuron	✓		Reserpine	✓	
Cimetadine	✓		Salbutamol	✓	
Clobetasol	×		Simazine	✓	
Cocaine	✓		Sparteine	×	
Colchizine	✓	weak	Tamoxifen	✓	
Dexamethasone	✓		Terfenadine	✓	
Diphenhydramine	✓		Thiabendazole	✓	weak
Disopyramide	✓		Thiphensulphuron methyl	×	
Erythromycin	✓	weak	Triflusulphuron methyl	✓	

succinate. The formation of erythromycin is more prevalent when organic acids are used as MALDI matrix (see Fig. 2(c), (i) and (ii)). The base peak, *m/z* 756, is attributed to $[M+Na]^+$ for erythromycin and reinforced by the presence of ions at *m/z* 734 and 772, which would correspond to protonated and potassiated erythromycin, respectively. Further the dehydration product of erythromycin, readily observed upon acid hydrolysis of solutions of erythromycin, can be seen; the

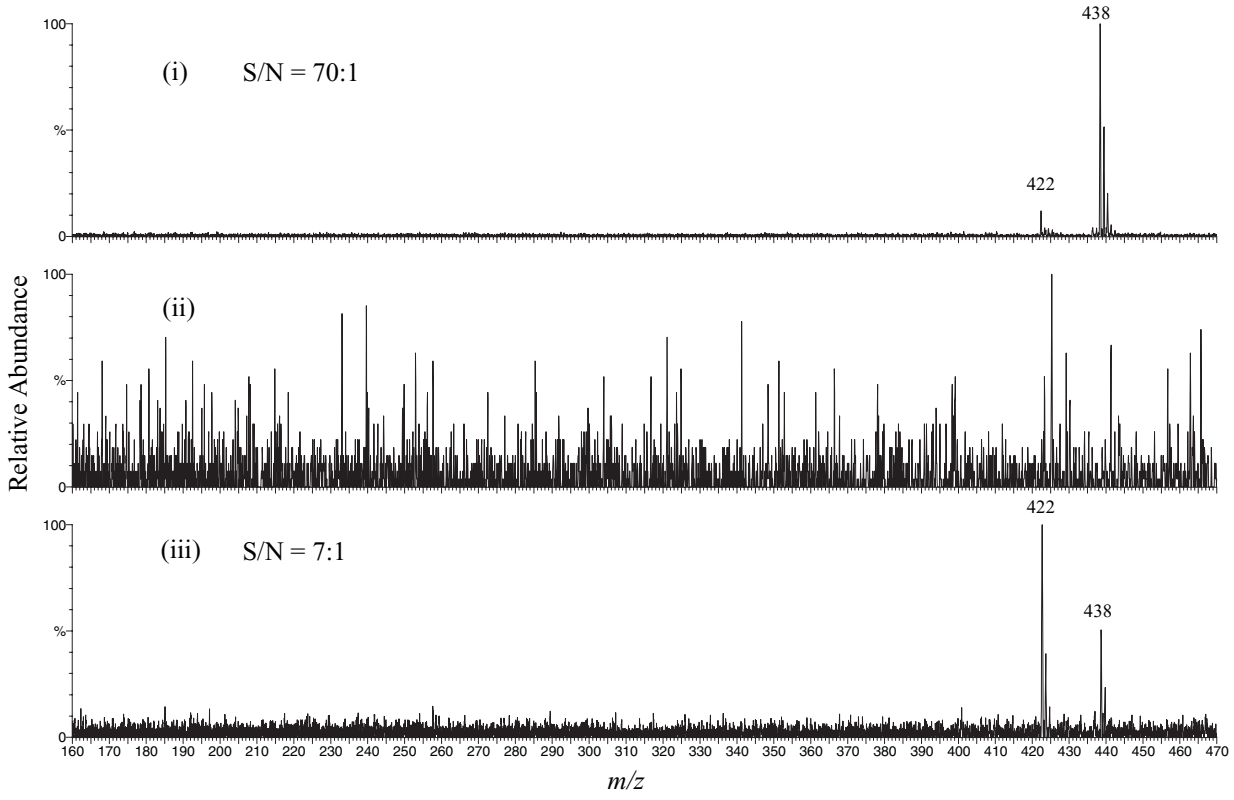

Figure 1. (i) MALDI-TOF mass spectrum of colchizine using 2B PL matrix at 50% coarse, 50% fine laser power; (ii) LDI-TOF mass spectrum of colchizine at 50% coarse, 50% fine laser power; and (iii) LDI-TOF mass spectrum of colchizine at 100% coarse, 100% fine laser power, where *m/z* 422 = $[M+Na]^+$ and *m/z* 438 = $[M+K]^+$.

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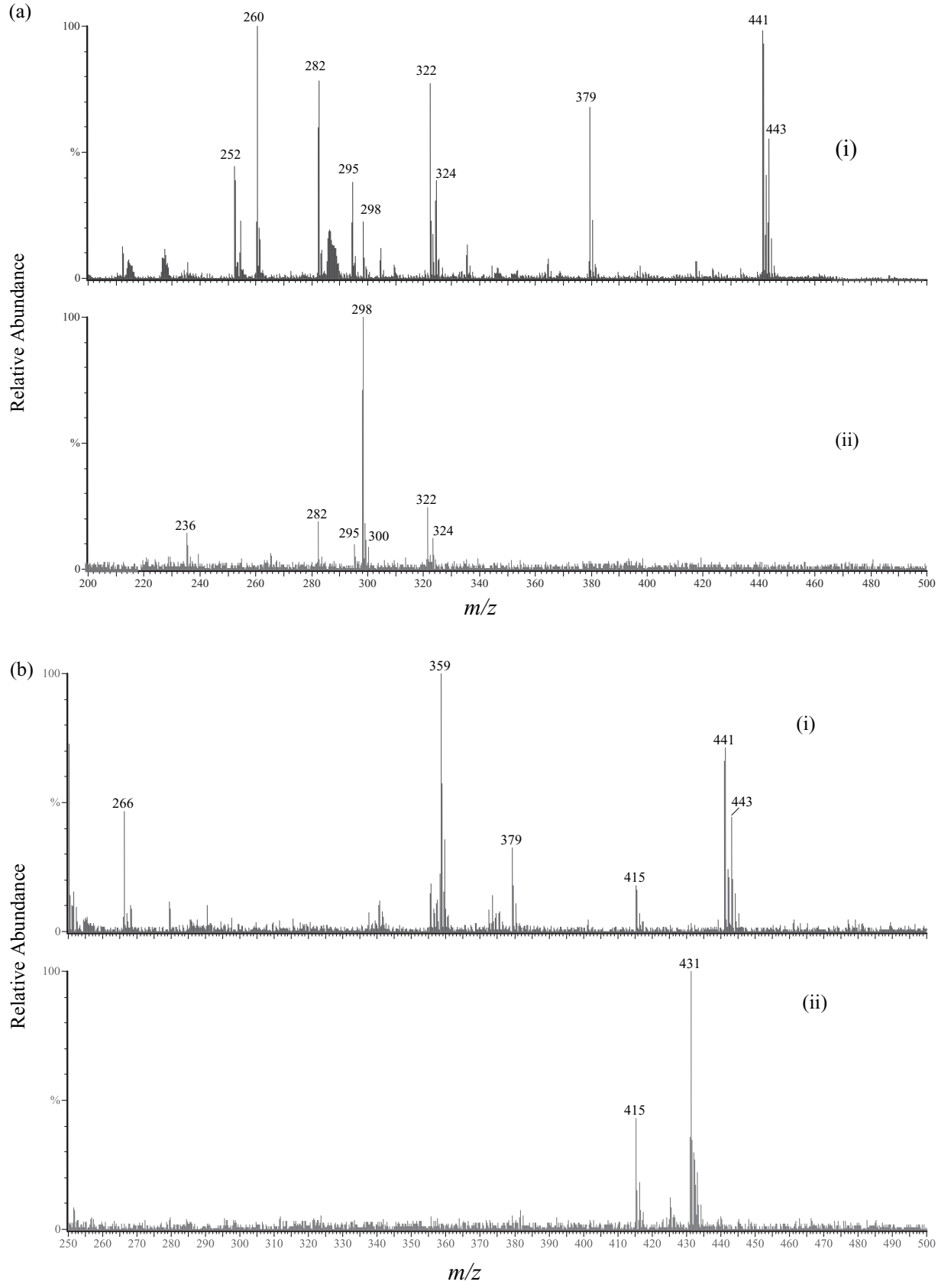

Figure 2.

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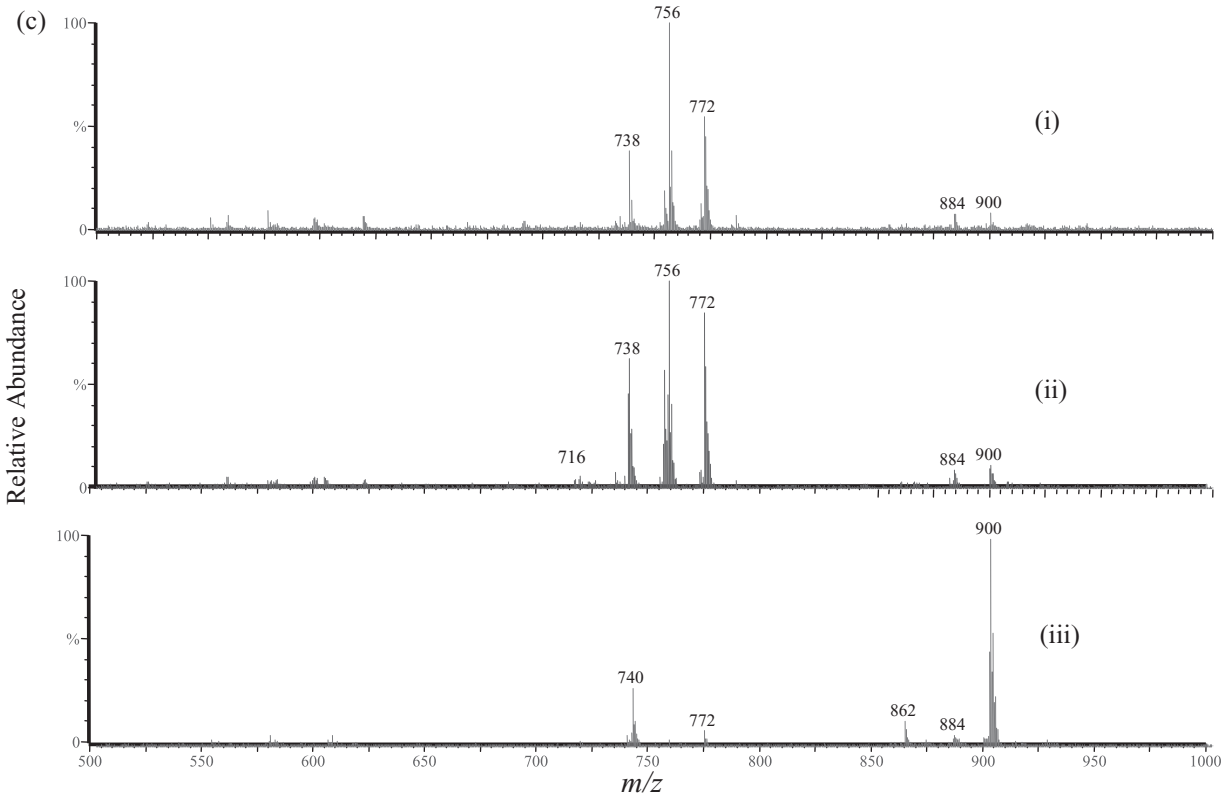

Figure 2. (a) MALDI-TOF mass spectra of Ala-Leu-Gly using (i) α-cyano-4-hydroxycinnamic acid (CHCA) with 0.1% formic acid and (ii) 2B PL matrix where *m/z* 282 = $[M+Na]^+$, *m/z* 298 = $[M+K]^+$ and *m/z* 322 = $[M+Cu]^+$. (b) MALDI-TOF mass spectra of dexamethasone using (i) CHCA with 0.1% formic acid and (ii) 2B PL matrix where *m/z* 415 = $[M+Na]^+$ and *m/z* 431 = $[M+K]^+$. (c) MALDI-TOF mass spectra of erythromycin ethyl succinate using (i) 2, 5-dihydroxybenzoic acid (DHB) with 0.1% formic acid; (ii) CHCA with 0.1% formic acid; and (iii) 2B PL matrix where *m/z* 884 = $[M+Na]^+$ and *m/z* 900 = $[M+K]^+$.

sodiated molecule is observed at *m/z* 738 with associated protonated and potassiated species at *m/z* 716 and 754, respectively. This example shows how the use of PL-MALDI aids compound identification and avoids possible side reactions that can be encountered with organic matrices.

Different brands and manufacturers of pencils use different materials in their manufacturing procedure in addition to pure graphite, i.e. different clays and glide agents. The role that these materials play in the MALDI process is unclear but preliminary experiments have suggested that the glide agents, i.e. the oils or waxes, aid deposition and adherence of the graphite to the MALDI target. This was reaffirmed where graphite rod (99.9%) was found to be an inferior graphite matrix source due to poor transfer and poor adhesion of graphite to the metal target.[15]

The glide agents vary from mainly mixtures of long-chain hydrocarbons (C12–18), these occasionally mixed with phthalates, to mixtures of hydrocarbons and long-chain acids. In the case of the latter, the saturated C16 and C18 carboxylic acids are observed in the PL-MALDI spectrum at *m/z* 295 and 323, respectively, for the potassiated molecules (see Fig. 3). The identity of these species has been confirmed by solvent extraction of the pencil lead followed by GC/EIMS (Fig. 4) and library matching of the EI mass spectra. Several brands and hardness of pencil were tested and spectra for selected GC peaks are shown in Fig. 5. The data shows that different brands and types of pencil contain different organic species used as glide agents and that the relative amount of these organics varies according to pencil type, grade and manufacturer. The 2B pencils provided by both Dixon® and Woolworths are dominated by a series of long-chain hydrocarbons and show no evidence for the presence of acids (see Figs. 4(i) and 4(ii)). The Niceday pencils, both 2B of differing densities, also contain the long-chain hydrocarbons as well as dibutyl phthalate, as shown by the peak at ~31.6 min in Figs. 4(iii) and 4(iv). The long-chain acids (hexadecanoic acid, octadecanoic acid and a corresponding oleate ester) dominate the chromatograms of all three Staedtler pencils, two types of 2B and one 6B (Figs. 4(v)–4(vii)), and the broad hump at the end of the chromatogram is believed to be a mixture of high molecular weight, unresolved long-chain hydrocarbons. High-resolution ESI accurate mass measurement of the Staedlter pencil extracts further confirmed the identification of the long-chain acids where the potassiated molecules gave *m/z* 295.0236 (measured mass error 0.7 ppm) and *m/z* 323.2349 (measured mass error 0.7 ppm), consistent with the elemental composition for hexadecanoic and octadecanoic acid, respectively.

Invariably when using pencil matrix the potassiated molecule is the dominant species with the sodiated molecule also observed at a lower abundance. Often the protonated molecule is absent or of very low abundance. These cations probably originate from the various clays used in the pencil

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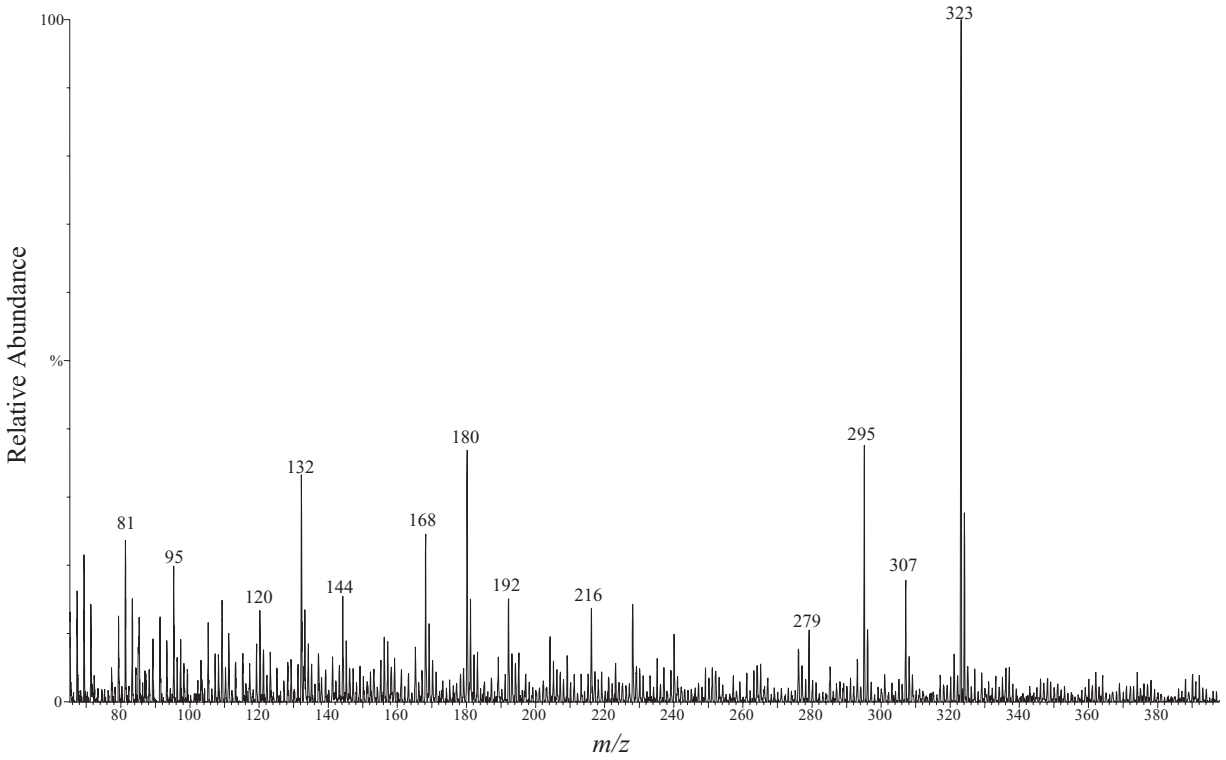

Figure 3. MALDI-TOF mass spectrum of PL matrix showing the presence of hexadecanoic acid $[M+Na]^+$ *m/z* 279 and $[M+K]^+$ *m/z* 295 with octadecanoic acid $[M+Na]^+$ *m/z* 307 and $[M+K]^+$ *m/z* 323.

manufacturing process, such as montmorillonite and illite. The presence of these cations simplifies the analysis of classes of compounds that usually require addition of cations to the sample/matrix mix to aid ionisation, e.g. some polymers and sugars. Notably some sugars have been successfully analysed using this method but the specific data cannot be reported here for confidentiality reasons.

Initial analysis of actinides proved successful using PL-MALDI[15] and the scope for the analysis of transition metals has now been expanded to cover the lanthanides.

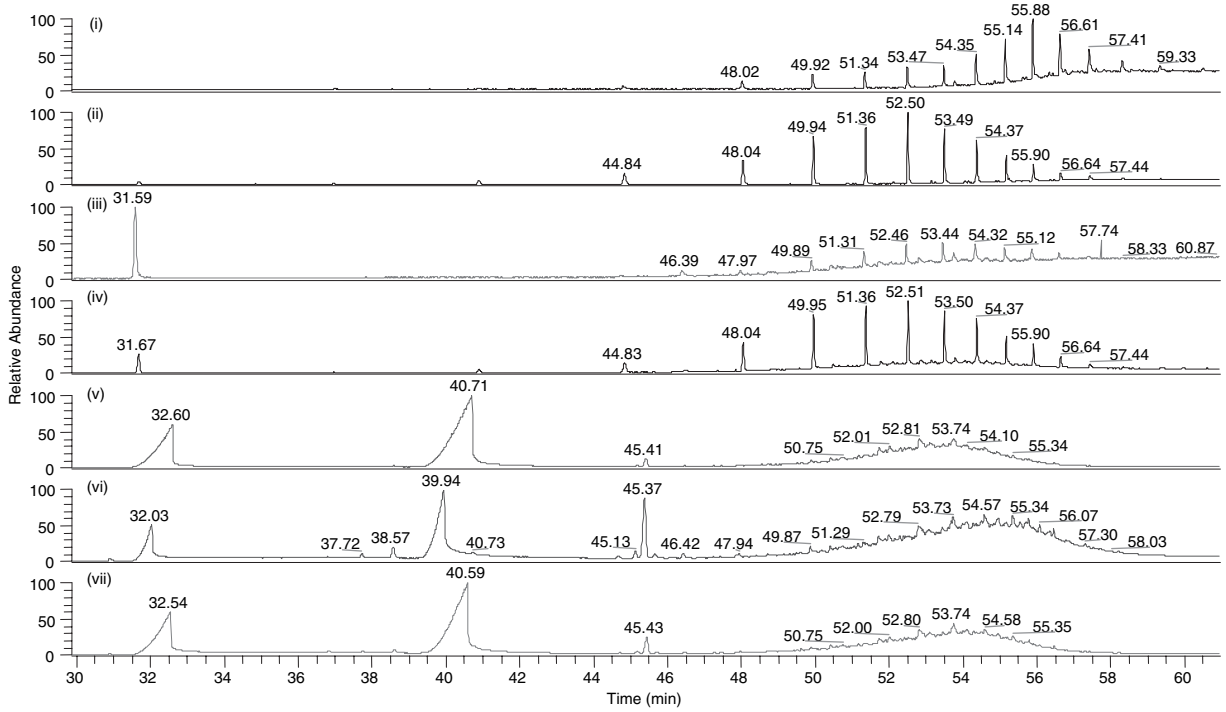

Figure 4. GC total ion current chromatograms of DCM solvent extracts of pencil lead from different makes and grades of pencil: (i) Dixon® 2B, (ii) Woolworths 2B, (iii) Niceday CGG 100 HB, (iv) Niceday CGG 75 HB, (v) Staedtler 2B, (vi) Steadtler Mars 2B, and (vii) Staedtler Mars 6B.

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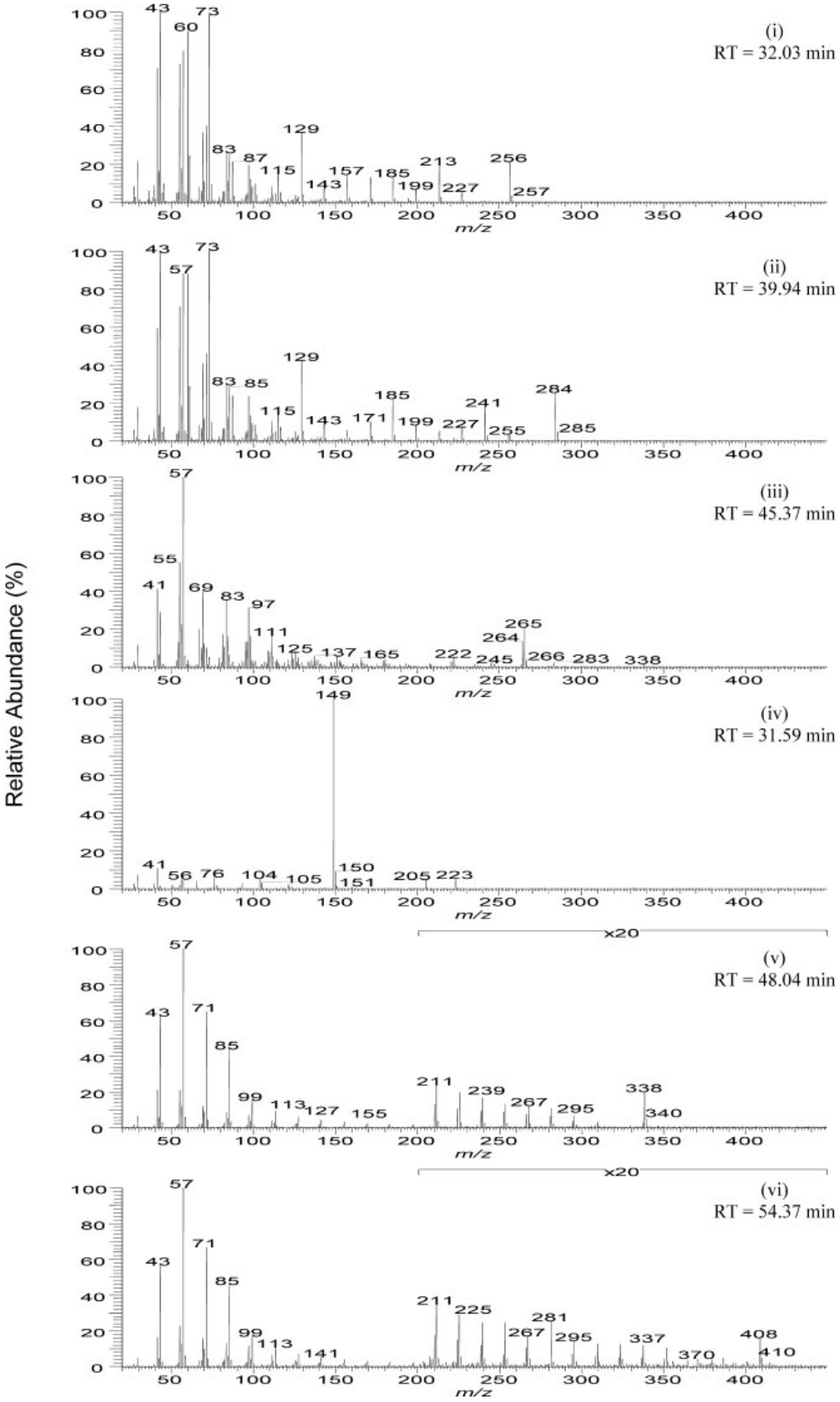

Figure 5. EI mass spectra of selected peaks from gas chromatograms: (i) hexadecanoic acid CAS#57-10-3, (ii) octadecanoic acid CAS#57-11-4, (iii) decyl oleate CAS#3687-46-5, (iv) dibutyl phthalate CAS#84-74-2, (v) tetracosane CAS#646-31-1, and (vi) tetratetracontane CAS#7098-22-8.

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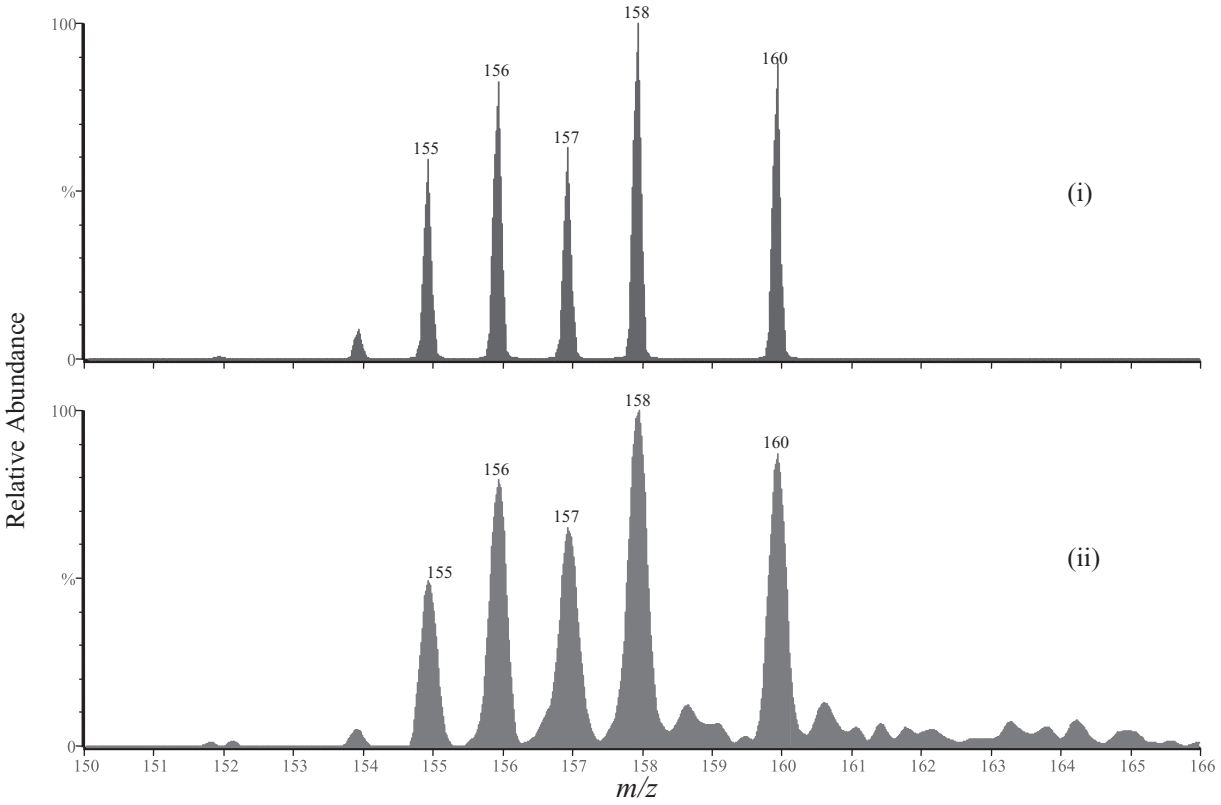

Figure 6. Theoretical isotope pattern (i) and MALDI-TOF mass spectrum (ii) of gadolinium ion from gadolinium nitrate using 2B PL matrix.

Dysprosium, erbium, gadolinium, lanthanum, praseodymium and ytterbium nitrate were all analysed successfully from the pencil matrix. The analysis is simpler and appears to be more efficient than for the corresponding actinides because it is easier to obtain data for the metal ion rather than the metal ion and its related oxide (as can be the case with the actinides if careful control is not taken with the laser fluence).[15] Previously, the actinides and lanthanides were not easily detected by MALDI when using standard organic matrices. However, the identification of the gadolinium metal ion is easily observed when using PL-MALDI at the standard laser power of 50% coarse and 50% fine, and the results compare favourably with the theoretical isotope pattern (Fig. 6).

Previously it was thought that for actinide analysis, the major requirement for a pencil to be a good matrix for analysis was simply a high graphite content, i.e. a high B number.[15] However, further analysis using different types of pencil here and in other laboratories has shown that the amount of graphite deposited on the MALDI plate surface plays a significant role in the quality of data recorded. GC/EIMS data has shown that pencils with the same grading contain different amounts and types of glide agents making the surface coverage variable and dependent upon pencil type. A further variable is the physical amount of material scribbled onto the target by the operator. If too much pencil is deposited onto the target, as can easily be achieved with 6B pencils, the ionisation process appears to be severely compromised resulting in poor S/N ratios being observed or, in the worst case, no analyte ions being recorded. In practice a 2B pencil has been shown to be a good choice since it routinely deposits a thin layer of pencil lead matrix onto the target surface. Further, use of this grade of pencil has shown that it is difficult to deposit excess pencil lead during matrix application (see Fig. 7).

To ensure that the pencil lead matrix did not give a one-hit use for the target plates, it was found that by following the simple three-step protocol the MALDI target plates could easily be cleaned and re-used with no carry-over of sample and an absence of plasticisers introduced in the cleaning procedure:

(1) Wash the target with the solvent used to deposit the sample and allow it to dry;
(2) Use a clean, dry pencil eraser and remove pencil from the target;
(3) Wash the target with methanol and/or acetone to remove residual eraser remnants and plasticisers.

Figure 8 shows the MALDI-TOF mass spectra of ranitidine analysed using PL matrix on both a new and a used and cleaned target plate. There is no evidence of sample carry-over or plasticiser contamination, notably dibutyl phthalate at *m/z* 301 ($[M+Na]^+$) and 317 ($[M+K]^+$) or dioctyl phthalate at *m/z* 413 ($[M+Na]^+$) and 429 ($[M+K]^+$).

All mass spectrometers require calibration and verification prior to analysis. In the case of a MALDI-TOF mass spectrometer either an external or internal mass calibration covering different mass ranges and modes of detection is essential, e.g. to address the topographical issue with the use of crystalline matrices. The development of the Cali-Pen was established to further improve analysis by providing a quick one-step calibration approach should it be deemed necessary

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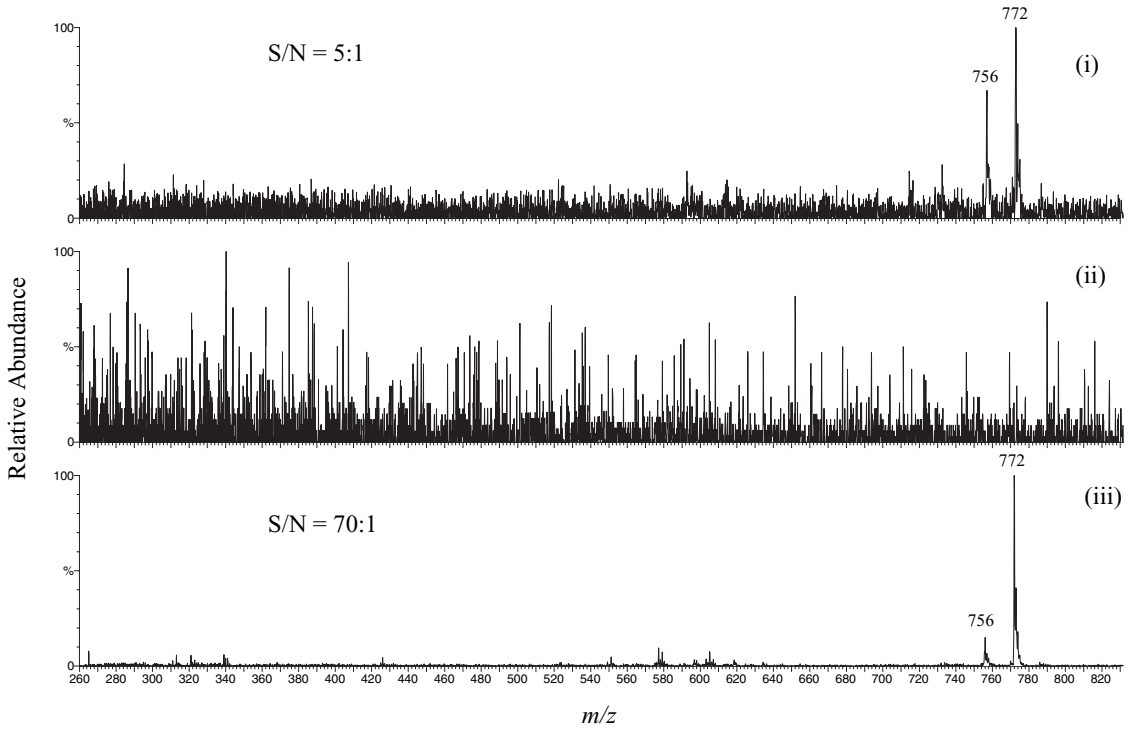

Figure 7. MALDI-TOF mass spectra of erythromycin with (i) insufficient PL matrix, e.g. HB pencil; (ii) excessive PL matrix, e.g. 6B pencil; and (iii) routine amount of PL matrix as deposited with a 2B pencil where *m/z* 756 = $[M+Na]^+$ and *m/z* 772 = $[M+K]^+$.

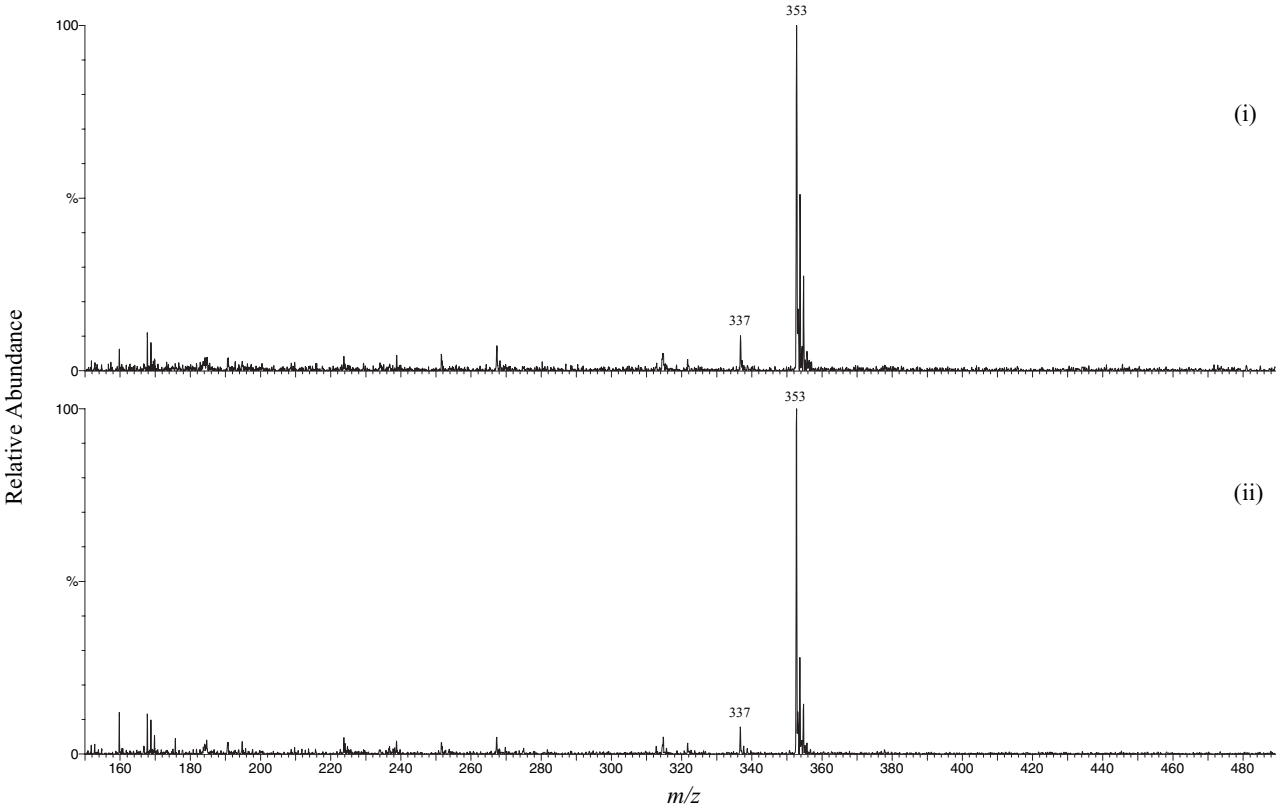

Figure 8. MALDI-TOF mass spectra of ranitidine using (i) 2B PL matrix on a new target plate and (ii) 2B PL matrix on a used and cleaned target plate where *m/z* 337 = $[M+Na]^+$ and *m/z* 353 = $[M+K]^+$.

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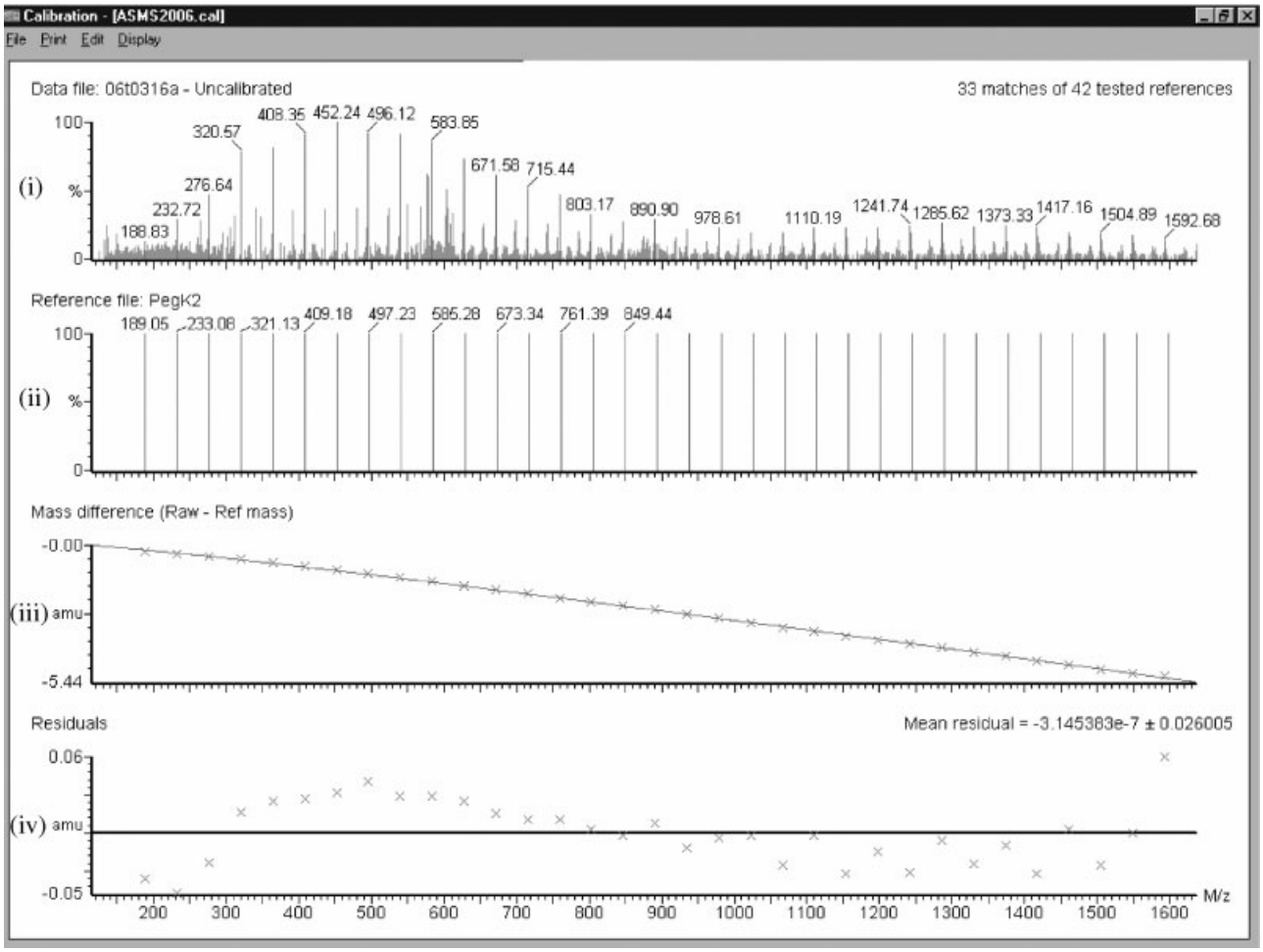

Figure 9. Mass calibration from a Cali-Pen showing (i) data file, (ii) reference file, (iii) linearity of calibration, and (iv) residual errors in mass calibration.

at any time during operation. Whilst the pencil itself can be used for mass calibration up to approx. *m/z* 300, pencils (previously used and shown to be good matrices) were impregnated with various calibration compounds. The appropriate pre-prepared Cali-Pen is then selected and scribbled onto the MALDI target as normal. Since the calibration compounds are already impregnated into the pencil at appropriate relative abundances, the time-consuming balancing of the ion abundances of each calibrant is avoided. In addition no drying time is required at this stage, and hence a quick instrument calibration and performance check can be achieved. Figure 9 shows the calibration data from a polyethylene glycol (PEG) Cali-Pen. This shows excellent, linear mass calibration over the mass range *m/z* 189.05–1597.89.

CONCLUSIONS

Pencil has proved to be a successful matrix for the analysis of small molecules. It is an additional and useful alternative matrix in the arsenal of MALDI matrices; one of its attractions being the simplicity of application. This ease of application and the lack of solvent compatibility issues, together with the absence of matrix ions in the low-mass range, make it the first choice matrix option for small molecule MALDI analysis. The range of compounds amenable to a pencil matrix for MALDI analysis has been extended from a limited number of small molecules and the actinides to a much broader range of molecules including, peptides, polymers, steroids and sugars. It has also proved to be particularly successful for the identification of the lanthanides especially if a specific grade of pencil is used. This could open up access to lower mass range MALDI analysis making it a useful tool for single bead analysis in combinatorial chemistry for low-mass analytes.[17]

The simple calibration procedure via a Cali-Pen offers simple sample preparation for verification of instrument performance. Further, the fact that the cationised species, $[M+Na]^+$ and $[M+K]^+$, are invariably observed from PL-MALDI aids molecular weight identification; the 16 *m/z* unit difference between the sodiated and potassiated species helps to determine the relative molecular mass of the molecules studied. If this 16 *m/z* unit difference is not observed a pre-charged species, e.g. a quaternary ammonium or metal (see lanthanide spectrum, Fig. 6), may be present.

The different hardness of pencils and the different manufacturing processes used by suppliers can influence the quality of PL-MALDI data. In the case of hard pencils too little graphite appears to be deposited onto the MALDI target, whereas it is possible to easily deposit too much

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material when using a soft, high graphite content pencil, e.g. 6B. Both these examples can result in poor or no ionisation of the sample. In practice a 2B pencil appears to give the optimum results for the molecules investigated in this study, i.e. 2B or not 2B was the question, and the results reported here suggest that 2B is the answer!

REFERENCES

1. Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T. *Rapid Commun. Mass Spectrom.* 1988; **2**: 151.
2. Karas M, Hillenkamp F. *Anal. Chem.* 1988; **60**: 2299.
3. Cohen LH, Gusev AI. *Anal. Bioanal. Chem.* 2002; **373**: 571.
4. Zhu YF, Lee KL, Tang K, Allman SL, Taranenko NI, Chen CH. *Rapid Commun. Mass Spectrom.* 1995; **9**: 1315.
5. Harvey DJ. *Mass Spectrom. Rev.* 1999; **18**: 349.
6. Nielen MWF. *Mass Spectrom. Rev.* 1999; **18**: 309.
7. Jones RM, Lamb JH, Lim CK. *Rapid Commun. Mass Spectrom.* 1995; **9**: 968.
8. Williams JB, Gusev AI, Hercules DM. *Macromolecules* 1996; **29**: 8144.
9. Armstrong DW, Zhang L-K, He L, Gross ML. *Anal. Chem.* 2001; **73**: 3679.
10. McCombie G, Knochenmuss R. *Anal. Chem.* 2004; **76**: 4990.
11. Sunner J, Dratz E, Chen Y-C. *Anal. Chem.* 1995; **67**: 4335.
12. Dale MJ, Knochenmuss R, Zenobi R. *Anal. Chem.* 1996; **68**: 3321.
13. Crecelius A, Clench MR, Richards DS, Parr V. *J. Chromatogr. A* 2002; **958**: 249.
14. Kin H-J, Lee J-K, Park S-J, Ro HW, Yoo DY, Yoon DY. *Anal. Chem.* 2000; **72**: 5673.
15. Black C, Poile C, Langley J, Herniman J. *Rapid Commun. Mass Spectrom.* 2006; **20**: 1053.
16. Petronski H. *The Pencil: A History of Design and Circumstance*. Knopf: New York, 1990.
17. Egner BJ, Langley GJ, Bradley M. *J. Org. Chem.* 1995; **60**: 2652.

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