

# Analysis of small organics on planar silica surfaces using surface-assisted laser desorption/ionization mass spectrometry

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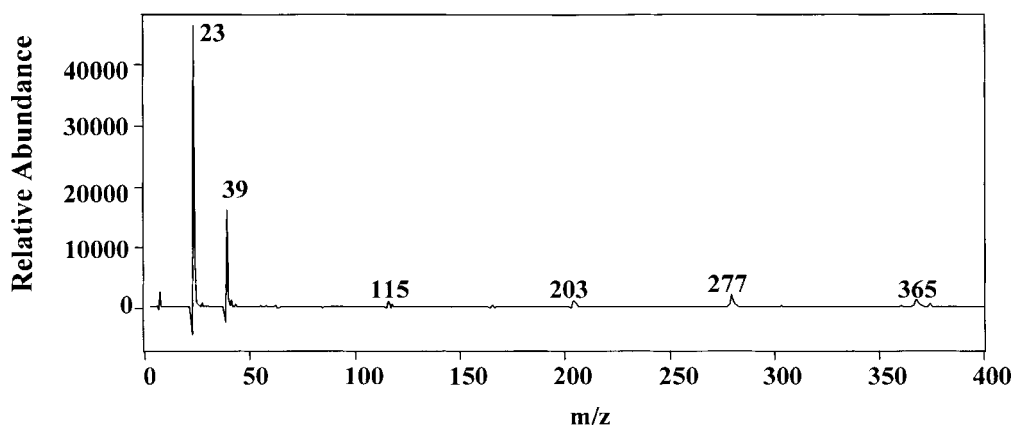
**This study demonstrates the feasibility of directly obtaining analyte signals from a planar silica gel surface by using laser desorption mass spectrometry (LDMS). The surface of the planar silica gel was pretreated using a line drawn by a soft carbon pencil, and 0.3  $\mu\text{L}$  of a liquid matrix (15% sucrose/glycerol-methanol 1:1, v/v) was applied to it (width  $\sim 1\text{mm}$ ) before sample deposition. The laser was then used to directly irradiate the sample spot on the pencil line ( $\sim 2\text{mm}$ ). Carbon powder ablated from the pencil residue is used to couple the laser UV energy into a liquid solution in LDMS. This sample preparation method is related to surface-assisted laser desorption/ionization (SALDI) mass spectrometry, which uses a suspension of  $\mu\text{m}$ -sized carbon powder (SALDI solid) mixed with a viscous liquid (SALDI matrix) as the matrix. In the present approach, the ablated carbon powder from the pencil drawing on a planar silica gel surface was used as an alternative to the SALDI solid. The liquid matrix was that used in conventional SALDI matrix systems, and the sample preparation applied herein is simpler than conventional SALDI. The matrix background remains quite low in this approach, which significantly assists the analysis of small organics such as methylephedrine and cytosine. PEG (polyethylene glycol) 400 and PEG 2000 were also used for demonstration purposes. Copyright © 2001 John Wiley & Sons, Ltd.**

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry has been extensively applied to analyze large biomolecules.<sup>1–5</sup> However, the application of laser desorption mass spectrometry to analyze small organics has also attracted attention.<sup>6–21</sup>  $\text{C}_{60}$  has been reported to be a good matrix for analytes with molecular masses between 200 and 600 Da, since a matrix-free window exists at this mass range in negative ion mass spectra.<sup>15</sup> Additionally, a method called surface-assisted laser desorption/ionization (SALDI) time-of-flight mass spectrometry has also been applied to analyze small organics.<sup>6–13</sup> The suspension of  $\mu\text{m}$ -sized carbon powder (graphite or activated carbon) in a liquid is used as the matrix in SALDI analysis.<sup>6–12</sup> The carbon powder is used to couple laser energy into the liquid solution. Relatively few (SALDI) matrix ions are produced in this method. Based on the characteristics of low matrix interference and the absorption capability of activated carbon, SALDI has been combined with other analytical methods such as thin layer chromatography<sup>7,9</sup> and solid phase extraction<sup>11,12</sup> to analyze small organics.

Matrix-free methods, such as desorption/ionization on porous silicon (DIOS-MS) facilitated by electrochemically treating silicon surfaces,<sup>16,17</sup> and mass analysis using silicon

films (MASiF) by making a silicon surface from plasma-enhanced chemical vapor deposition,<sup>18</sup> have recently been successfully applied for the analysis of small organics. Wei *et al.* also observed that use of 60 Å pore silica gel thin layer chromatography (TLC) plates as a platform for sample deposition produced no significant background ion signals in laser desorption/ionization mass spectra;<sup>16</sup> indeed, no analyte signals were obtained from the TLC plate unless the silica gel surface was pre-treated. Our previous work noted that only trace amounts of carbon powder ablated from the pencil drawing, normally present on the TLC chromatographic spots for purposes of identification, helped in obtaining analyte signals.<sup>9</sup> Based on this observation, the ablation of carbon powder deposited on the silica gel plate from a pencil drawing can be used as an alternative to the SALDI solid, i.e. carbon powder. This approach still requires a liquid solution, and thus  $\sim 0.3\ \mu\text{L}$  of a glycerol/methanol solution was applied to the pencil line on the silica TLC plate before deposition of the sample solution. The ion signals are easily obtained along the track of the pencil line, and the treatment of the planar silica gel surface is thus simplified to suit the analytical requirements of laser desorption mass spectrometry. The experiment can be easily performed without requiring additional equipment to modify the planar silica gel surface. Furthermore, concerns about contamination of the ion source by the carbon powder from the suspension used in a conventional SALDI matrix can be eliminated, since only trace amounts of carbon powder were

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**Figure 1.** SALDI mass spectrum of 0.3  $\mu$ L of liquid matrix (15% sucrose/glycerol in methanol (1:1, v/v)) on the pencil line on a TLC plate.

ablated from the pencil drawing and deposited on the gel surface. Herein, we report on the new sample preparation method for SALDI analysis, and also investigate the characteristics of this approach.

## EXPERIMENTAL

### Reagents

Glycerol, polyethylene glycol (PEG) 400 and 2000 were purchased from Riedel-de Haen (Deisenhofen, Germany), and methylephedrine was obtained from Aldrich (Milwaukee, WI, USA). Cytosine was purchased from Sigma (St. Louis, MO, USA), sucrose from J. T. Baker (Phillipsburg, NJ, USA), and methanol from Mallinkrodt (Phillipsburg, NJ, USA). The 2B soft graphite pencil was purchased from Bentsia (USA).

### Sample preparation

A 0.5  $\times$  0.5 cm piece of TLC plate (Merck, Germany) was cut out using scissors. A pencil line approximately 0.5 mm wide was drawn on the silica gel plate. The cut-out piece was attached to the sample tray using double-sided tape. Meanwhile, 0.3  $\mu$ L of liquid solution, comprising 15% sucrose/glycerol in methanol (v/v = 1/1, referred to here as the SALDI liquid), was applied to the pencil line on the piece of the TLC plate. Sucrose was added to the glycerol solution for the purpose of enhancement of the viscosity of the liquid and as the internal standard for mass calibration. Subsequently, 0.3  $\mu$ L of sample solution was deposited in one spot on the pencil line. The diameter of the liquid spreading on the TLC plate was approximately 1 mm. The sample tray was loaded into the mass spectrometer after standing at room temperature for 5 min. Methylephedrine (3 mg/mL) and PEG 2000 were prepared in methanol, while cytosine (2.5 mg/mL) was prepared in water.

### Mass spectrometer

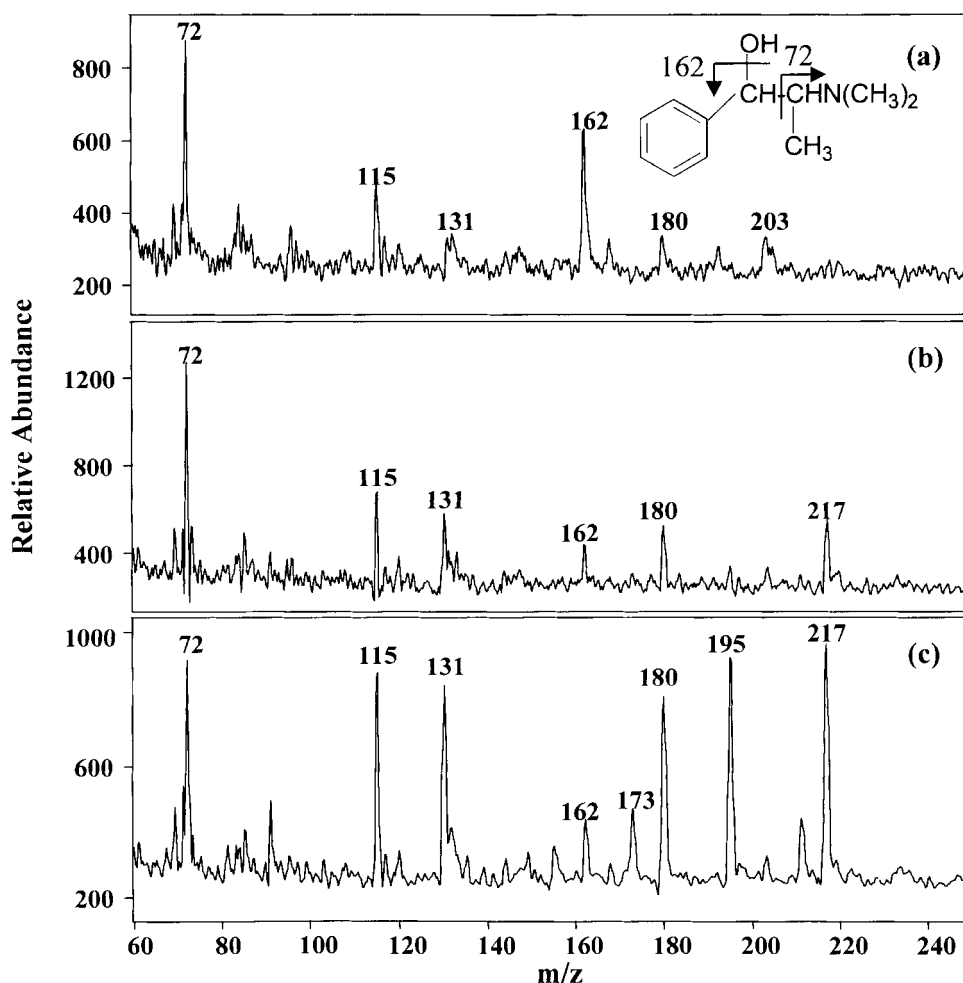
A Voyager linear time-of-flight (TOF) mass spectrometer (PerSeptive Biosystems, Framingham, MA, USA) was used, and this instrument was equipped with a 1.2 m linear flight tube and a 3 ns pulse width 337 nm nitrogen laser for desorption. The acceleration voltage was set to 20 kV. This

mass spectrometer uses a horizontal sample tray. The overall mass resolution of the mass spectra presented here was low ( $\sim$ 200) since linear TOF was used without delayed extraction. The ions obtained from the carbon at each single point only lasted a few seconds, and the intensity of the analyte ions decayed afterwards. Thus, the mass spectra were obtained by moving the laser back and forth along the carbon track ( $\sim$ 2 mm) in order to accumulate enough ions. All mass spectra were averaged over 64 laser shots. The laser power used in this study is about 10% higher than that normally used in MALDI.

## RESULTS AND DISCUSSION

This study focuses on the analysis of small organics by laser desorption mass spectrometry. Thus, a method that provides a clean matrix background over a low mass range is applied. Figure 1 shows the mass spectrum obtained by laser irradiating the pencil line on the TLC plate with 0.3  $\mu$ L of SALDI liquid deposition, and reveals a few ions contributed by the matrix. The two most intense peaks correspond to sodium and potassium ions at  $m/z$  23 and 39, respectively. The peak at  $m/z$  115 is sodiated glycerol, and that at  $m/z$  203 is the sodium ion adduct of a monosaccharide fragment. Furthermore, the peak at  $m/z$  277 is a fragment from sucrose, while that at  $m/z$  365 is sodiated ion of sucrose. If the laser failed to irradiate the sample spots on the track of the pencil line, no analyte signals could be obtained. This approach thus requires both the carbon powder ablated from the pencil drawing and the liquid matrix.

Figure 2(a) displays the SALDI mass spectrum of methylephedrine ( $MH^+$ ,  $m/z$  180) obtained from the planar silica gel surface of a TLC plate. Some fragment ions from methylephedrine are observed in this mass spectrum. Notably, the fragment at  $m/z$  162 ( $[MH^+ - H_2O]$ ) is observed in this spectrum, while the peak at  $m/z$  72 ( $[MH^+ - C_6H_5CH_2OH]$ ) is another fragment from methylephedrine. The peaks at  $m/z$  115 and 131 are the sodium and potassium adduct ions of glycerol, respectively. When the track of the pencil line on the TLC plate did not contain the liquid matrix, only the sodium and potassium ions were obtained in low yield.



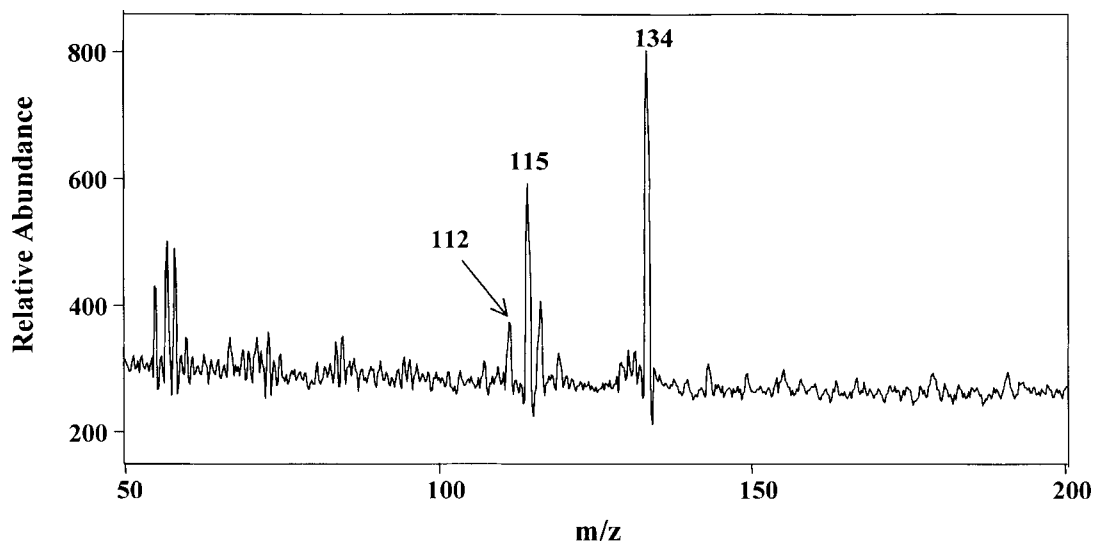
**Figure 2.** SALDI mass spectra of methylephedrine ( $MH^+$  at  $m/z$  180) with the deposition of  $0.3 \mu\text{L}$  of SALDI liquid (15% sucrose/glycerol in methanol (1:1, v/v)) on the track of the pencil line on the silica gel surface of a piece of a TLC plate. SALDI liquid contained (a) 0 M, (b) 0.5 M, and (c) 3 M of PTSA.

This method caused more fragmentation than conventional SALDI sample preparation.<sup>10</sup> The ionization process employing this sample preparation on the silica gel surface thus appears 'harder' than that in conventional SALDI although the sample preparation is easier. However, addition of extra additives into the SALDI matrix to protonate analyte molecules was found to stabilize the molecular structure and reduce fragmentation. It has been reported that *p*-toluenesulfonic acid (PTSA) can be used to enhance the analyte signals in SALDI analysis, owing to its characteristics as surfactant and acid.<sup>10</sup> PTSA was applied here to study the change in the intensity of the  $MH^+$  ion of methylephedrine. Figures 2(b) and 2(c) show the mass spectra of methylephedrine with PTSA concentrations of 0.5 M and 3 M spiked into the SALDI matrix, respectively. The ratio of the relative intensity of  $MH^+$  at  $m/z$  180 to  $[MH^+ - H_2O]$  at  $m/z$  162 in Fig. 2(b) exceeds that in Fig. 2(a), and the ratio in Fig. 2(c) is even higher than those in Figs. 2(a) and 2(b). Furthermore, the ratio of the relative intensity of the fragment ion at  $m/z$  72 to the  $MH^+$  ion at  $m/z$  180 in Fig. 2(c) is lower than in Fig. 2(a). Additionally, the ion peaks such as  $m/z$  173 (protonated PTSA ion =  $[PTSAH]^+$ ),  $m/z$  195 and 217 (sodiated PTSA ions =  $[PTSANa]^+$ ,  $[(PTSA-H)Na_2]^+$ )

that appeared in the mass spectra, resulted from elevated PTSA concentrations. These analytical results indicate that a suitable addition of PTSA to the SALDI matrix reduces the fragmentation of methylephedrine. The branch chain ( $-\text{CH}(\text{OH})\text{CH}(\text{CH}_3)\text{N}(\text{CH}_3)_2$ ) on the aromatic ring appears to have been stabilized by the protonation. The detection limit of methylephedrine is about 30 ng based on the present preliminary analytical results, defined in terms of a signal to noise ratio of 3.

Figure 3 displays the SALDI mass spectrum of cytosine ( $M_cH^+$  at  $m/z$  112) directly irradiated by a laser from the pencil line on the silica gel surface of a TLC plate. Sodiated cytosine ( $M_cNa^+$  at  $m/z$  134) is dominant in the mass spectrum. High percentages of sodium ion adducts are frequently observed to be generated by this approach.

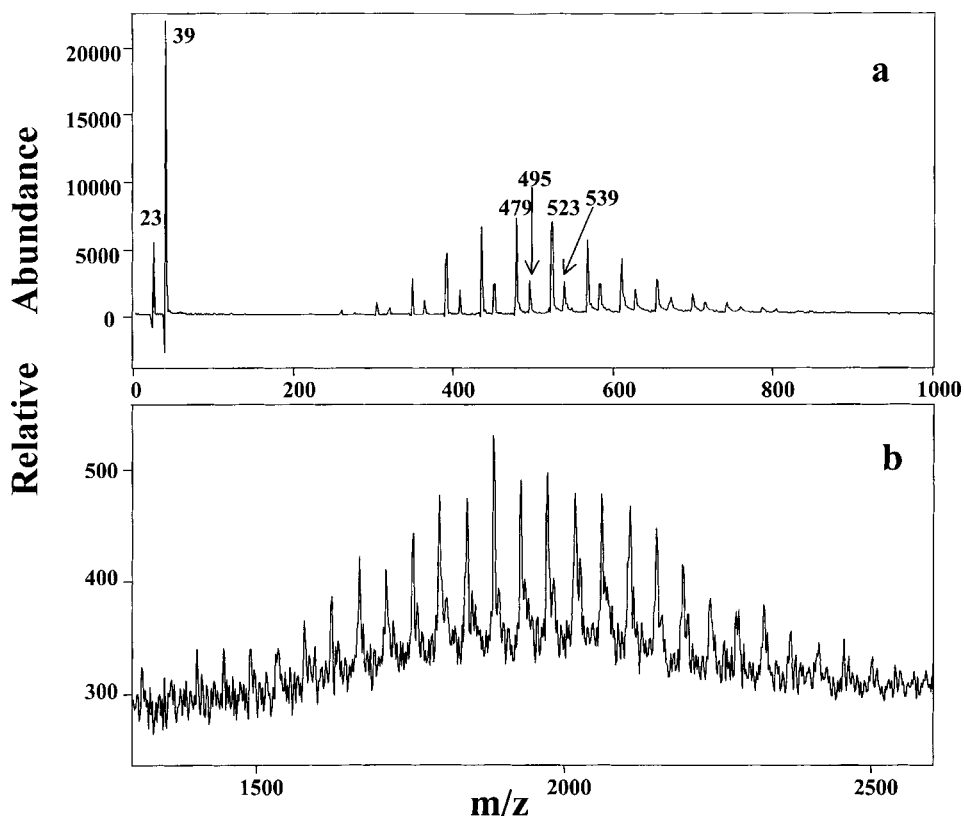
Besides these small organics, this method can be applied to analyze polymers. Polymers with low molecular weights, such as PEG 400, are viscous liquids at room temperature. These polymers can be applied to the pencil line on the TLC plate for direct SALDI analysis without requiring extra SALDI liquid. Figure 4(a) shows the SALDI mass spectrum of PEG 400 irradiated from the pencil line on the silica gel surface of a TLC plate, and contains two groups of ions. One



**Figure 3.** SALDI mass spectrum of cytosine ( $MH^+$  at  $m/z$  112) with the deposition of 0.3  $\mu$ L of SALDI liquid (15% sucrose/glycerol in methanol (1:1, v/v)) on the track of the pencil line on the silica gel surface of a piece of a TLC plate.

group is a series of sodiated PEG 400 ions, while the other series is the potassium adduct ions. The adjacent peaks from these two groups have a mass difference of 16 Da. Besides these ions, only the bare sodium and potassium ions appear at the low mass range. However, polymers with higher

molecular weights, such as PEG 2000, are solid at room temperature and require the assistance of a SALDI liquid to generate the ion signals in laser desorption/ionization. Figure 4(b) shows the SALDI mass spectrum of PEG 2000 irradiated from the silica gel surface of a TLC plate. Unless



**Figure 4.** (a) SALDI mass spectrum of PEG 400 irradiated from the pencil line on a silica gel TLC plate without the deposition of extra SALDI liquid. (b) SALDI mass spectrum of PEG 2000 irradiated from a pencil line on the silica gel surface of a TLC plate with the deposition of 0.3  $\mu$ L of SALDI liquid (15% sucrose/glycerol in methanol (1:1, v/v)).

the SALDI liquid is deposited on the pencil line, no analyte signal can be observed in the SALDI mass spectrum. This phenomenon explains the significance of the presence of SALDI liquid in this sample preparation. The SALDI liquid is thus proposed to be a buffer medium for preventing deposition of excessive laser energy in the analyte molecules, which may destroy the molecules before the occurrence of desorption/ionization. The analyte ions can still be observed after standing in the mass spectrometer for half an hour. Since the SALDI liquid is essential to assist the desorption/ionization process, the useful lifetime of the SALDI liquid is about half an hour.

## CONCLUSIONS

Use of ablated carbon powder from soft carbon pencil drawing on the silica gel surface of a TLC plate as the SALDI solid has simplified sample preparation in SALDI analysis. Treating the silica gel surface by simply drawing a pencil line is very easy, and no complicated procedures or equipment are required for successful analysis. Additionally, interference from the matrix is very low. Adding extra additives to the SALDI liquid may improve detection limits, and other additives like PTSA are being sought for the above purpose.

The present method is suited to analysis of small organics. The sample is directly applied to the TLC plate, and the method should be suitable for combining TLC with SALDI. The combination of TLC with LDMS by using the conventional SALDI matrix system has been reported previously. Further investigations of the applicability of the novel method to the TLC-LDMS combination are currently underway.

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## REFERENCES

1. Karas M, Hillenkamp F. *Anal. Chem.* 1988; **60**: 2299.
2. Solouki T, Marto JA, White FM, Guan S, Marshall AG. *Anal. Chem.* 1995; **67**: 4139.
3. Kussmann M, Lässig U, Stürmer CAO, Przybylski M, Roepstorff P. *J. Mass Spectrom.* 1997; **32**: 483.
4. Sze ET, Chan TW, Wang G. *J. Am. Soc. Mass Spectrom.* 1998; **9**: 166.
5. Kingshott P, St John HA, Chatelier RC, Griesser HJ. *J. Biomed. Mater. Res.* 2000; **49**: 36–42.
6. Sunner J, Dratz E, Chen Y-C. *Anal. Chem.* 1995; **67**: 4335.
7. Chen Y-C, Shiea J, Sunner J. *J. Chromatogr. A* 1998; **826**: 77.
8. Kraft P, Alimpiev S, Dratz D, Sunner J. *J. Am. Soc. Mass Spectrom.* 1998; **9**: 912.
9. Chen Y-C. *Rapid Commun. Mass Spectrom.* 1999; **13**: 821.
10. Chen Y-C, Tsai M-F. *J. Mass Spectrom.* 2000; **35**: 1278.
11. Chen Y-C, Shiea J, Sunner J. *Rapid Commun. Mass Spectrom.* 2000; **14**: 86.
12. Chen Y-C, Tsai M-F. *Rapid Commun. Mass Spectrom.* 2000; **14**: 2300.
13. Han M, Sunner, J. *J. Am. Soc. Mass Spectrom.* 2000; **11**: 644.
14. Dale MJK, Knochenmuss R, Zenobi R. *Anal. Chem.* 1996; **68**: 3321.
15. Huang J-P, Yuan C-H, Shiea J, Chen Y-C. *J. Anal. Toxicol.* 1999; **23**: 337.
16. Wei J, Buriak JM, Siuzdak G. *Nature.* 1999; **399**: 243.
17. Shen Z, Thomas JJ, Averbuj C, Broo KM, Engelhard M, Crowell JE, Finn MG, Siuzdak G. *Anal. Chem.* 2001; **73**: 612.
18. Cuijffi JD, Hayes DJ, Fonash SJ, Brown KN, Jones AD. *Anal. Chem.* 2001; **73**: 1292.
19. Zhang Q, Zou H, Guo Z, Zhang A, Chen X, Ni J. *Rapid Commun. Mass Spectrom.* 2001; **15**: 217.
20. Ayorinde FO, Hambright P, Porter TN, Keith QL Jr. *Rapid Commun. Mass Spectrom.* 1999; **13**: 2472.
21. Kinumi T, Saisu T, Takayama M, Niwa H. *J. Mass Spectrom.* 2000; **35**: 416.