Tumor Detection

Deutsche Ausgabe: DOI: 10.1002/ange.201502770
Internationale Ausgabe: DOI: 10.1002/anie.201502770

In Vivo Endoscopic Tissue Identification by Rapid Evaporative Ionization Mass Spectrometry (REIMS)

*Julia Balog, Sacheen Kumar, James Alexander, Ottmar Golf, Juzheng Huang, Tom Wiggins, Nima Abbassi-Ghadi, Attila Enyedi, Sandor Kacska, James Kinross, George B. Hanna, Jeremy K. Nicholson, and Zoltan Takats**

Abstract: *Gastrointestinal cancers are a leading cause of mortality, accounting for 23 % of cancer-related deaths worldwide. In order to improve outcomes from these cancers, novel tissue characterization methods are needed to facilitate accurate diagnosis. Rapid evaporative ionization mass spectrometry (REIMS) is a technique developed for the in vivo classification of human tissue through mass spectrometric analysis of aerosols released during electrosurgical dissection. This ionization technique was further developed by utilizing surface induced dissociation and was integrated with an endoscopic polypectomy snare to allow in vivo analysis of the gastrointestinal tract. We tested the classification performance of this novel endoscopic REIMS method in vivo. It was shown to be capable of differentiating between healthy layers of the intestinal wall, cancer, and adenomatous polyps based on the REIMS fingerprint of each tissue type in vivo.*

Despite increasing incidence, mortality from cancer has generally been decreasing over the last four decades.[1,2] Endoscopy represents a core diagnostic tool for the early detection of gastrointestinal (GI) tumors, and it is routinely deployed in national screening programs that have played an important role in reducing the burden of this disease.[3] Conventional white-light endoscopic investigation of the GI tract with tissue biopsy is the gold standard method for the diagnosis of GI cancers.[4] However, up to 7.8 % of upper-GI cancers may be missed by this technique in patients who are subsequently diagnosed with cancer.[5] Moreover, there is a significant economic and service burden on histopathology specialists who have to report on benign polyps, which may be found in 13.5 to 75 % of colonoscopies.[6] A major advantage of endoscopic intervention is that it provides a less invasive therapeutic option compared to conventional open surgical approaches. Early-stage cancers and premalignant conditions are now routinely treated using endoscopic mucosal resection (EMR) techniques that utilize electrosurgical diathermy and radiofrequency ablation (RFA).[7] However, re-intervention is necessary in up to 41 % of patients owing to incomplete excision.[8] Hence, there remains a need to develop accurate in situ techniques for improving the adenoma detection rate (ADR) and for real-time chemical biopsies that can detect dysplasia or cancer in vivo to increase the efficacy and safety of endoscopic therapies.

Enhanced endoscopic imaging technologies are currently being developed to address these requirements, with a specific emphasis on spectroscopic characterization using elastic scattering spectroscopy, optical coherence tomography, and multimodal imaging combining Raman spectroscopy, autofluorescence, and narrow band imaging.[9] However, optical strategies do not provide detailed information on tumor biology or chemistry and cannot be deployed during therapeutic procedures to provide data on cancer-margin status. Mass spectrometry (MS)-based identification of tissues has been employed in the context of MS imaging, probe electrospray, and the direct ambient ionization MS investigation of tissues.[10] Rapid evaporative ionization MS (REIMS) has emerged from this latter group as a technique that enables in vivo, in situ, real-time analysis through the utilization of electrosurgical tools as MS ion sources. The REIMS fingerprint of human tissues shows high histological specificity, with 90–100 % concordance compared to standard histology,[11] and it overcomes many of the shortcomings of optical biopsy. The aim of the current study was to develop a novel REIMS-based technology for real-time robust endoscopic tissue characterization.

The challenges in developing REIMS-based endoscopic methods include the short signal-capture window (1–2 seconds), aspiration of aerosol from a closed cavity, potential contamination from the GI tract, and the need for a long sampling line (> 4 m). To address these issues, the endoscopic setup was optimized and reproducibility assessed in a food-grade porcine stomach model. Artificial polyps were created within the stomach mucosa and resections were undertaken using a polypectomy snare (Figure 1 B); this setup allows exact simulation of a standard endoscopic resection. The resultant aerosol was aspirated through fenestrations created on the plastic sheath (Figure 1 B). These aerosol particles were transferred to the mass spectrometer via PFTE tubing connected to the irrigation port of the snare on the proximal

[*] Dr. J. Balog,[+] O. Golf, J. Kinross, Prof. J. K. Nicholson, Prof. Z. Takats
Computational and Systems Medicine, Imperial College London
South Kensington Campus, London SW7 2AZ (UK)
E-mail: z.takats@imperial.ac.uk
Dr. S. Kumar,[+] J. Alexander, Dr. F. J. Huang, T. Wiggins,
N. Abbassi-Ghadi, Prof. G. B. Hanna
Department of Surgery and Cancer, Imperial College London
St. Mary's Hospital, QEQM 10th floor, London W2 1NY (UK)
Dr. A. Enyedi
Institute of Surgery, University of Debrecen
Moricz Zsigmond krt. 22, Debrecen 4032 (Hungary)
Dr. S. Kacska
Gastroenterology Clinic, University of Debrecen
Nagyerdei krt. 98., Debrecen 4032 (Hungary)
[+] These authors contributed equally to this work.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201502770.

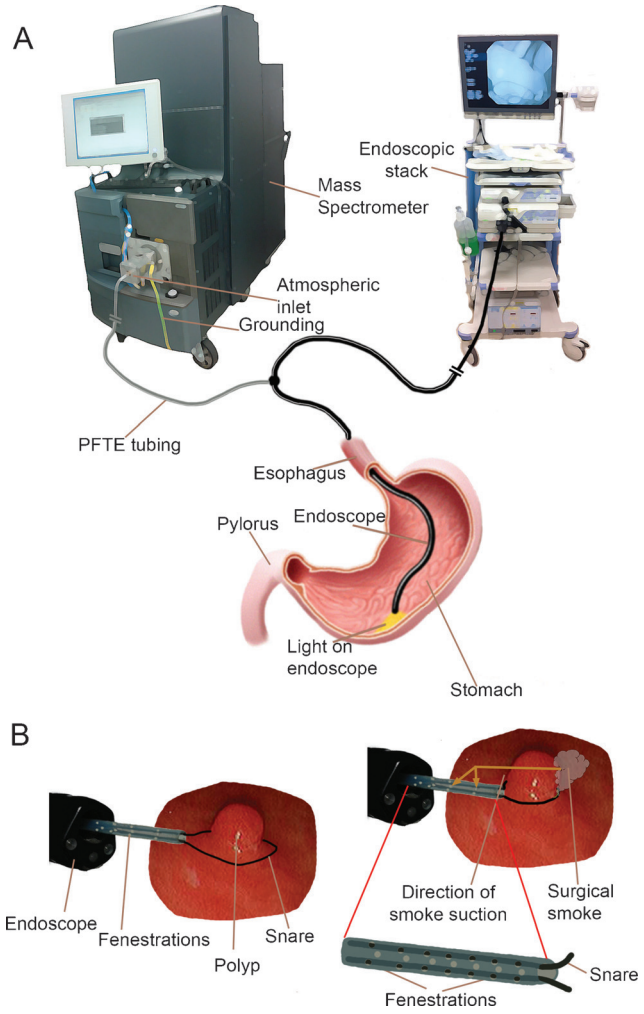

Figure 1. Endoscopy experimental setup. A) The polypectomy snare was equipped with an additional T-piece in order to establish direct connection between the electrode tip and the mass spectrometer for the transfer of electrosurgical aerosol. B) Resection of GI polyps by using a commercial snare. The polyp is captured with the snare loop, which is tightly fastened around its base. Electrosurgical dissection is performed and the generated aerosol is aspirated through the fenestrations created on the plastic sheath of the snare.

end, and directly to the inlet capillary of the mass spectrometer on the distal end (Figure 1 A).

The modification of the MS instrument included the development of a new atmospheric interface design featuring a heated jet disruptor element (heated metal coil) positioned into the central axis of the large opening of the Stepwave ion guide (Figure S1 in the Supporting Information). The jet disruptor surface facilitates fragmentation of molecular clusters formed in the free jet region owing to the adiabatic expansion of gas entering the vacuum and the resulting drop in temperature. The surface-induced dissociation of supramolecular clusters improves the signal intensity and also alleviates the problems associated with the contamination of ion optics. Since all aerosol particles or droplets of liquid aspirated from the gastrointestinal lumen undergo collisions with the disruptor surface, which is kept at 800 °C operating temperature, no macroscopic particulate matter can enter the instrument. Deposited organic material is subsequently incinerated, thus keeping the interface unchanged for up to several hours of operating time. When using the described experimental setup, a large percentage (> 95 %) of the formation of detected ions was associated with the collision phenomenon, which puts REIMS (and presumably a number of other ambient ionization MS methods) into new context regarding ionization mechanisms involving surface interactions.

REIMS spectra recorded from the porcine stomach model (Figure S2) in the range m/z 600–1000 features predominantly phospholipids, which have been observed for all mammalian tissue types in previous REIMS studies.[11,12] Initial experiments included optimization of the snare tip geometry (number and position of fenestrations on the plastic sheath) and an assessment of the repeatability of the analysis (see the Supporting Information).

Following optimization of the sampling geometry (Figure S3), the REIMS endoscopic setup was tested on ex vivo human samples including gastric adenocarcinoma, healthy gastric mucosa, and healthy gastric submucosa. These samples were acquired from three individual patients, all of whom provided written, informed consent. Our previous studies have demonstrated differences in the REIMS fingerprint of healthy mucosa and cancers from the GI tract;[11] however this is the first time that healthy submucosa and GI polyps have also been investigated by using an endoscope-compatible REIMS setup with a polypectomy snare.

Spectral differences were observed between healthy gastric mucosa, healthy gastric submucosa, and gastric cancer tissue. The spectra for healthy gastric mucosa (n = 32) and gastric adenocarcinoma (n = 29) featured phospholipids in the range m/z 600–900; whilst the gastric submucosa (n = 10) featured intensive triglyceride (TG) and phosphatidylinositol (PI) species in the m/z 850–1000 range (Figure 2 A). The submucosa in the GI tract represents a connective tissue layer containing arterioles, venules, and lymphatic vessels; it consists of mostly collagenous and elastic fibers with varying amounts of adipose elements. It is hypothesized that the PI and triglyceride species observed in the mass range m/z 850–1000 are associated with the histological features present within the submucosa. An interesting feature was observed regarding the abundance of phosphatidylethanolamines (PEs) and corresponding plasmalogen species. While the PEs show higher abundance, the plasmalogens are depleted in the tumor tissue, probably owing to the impaired peroxisomal function of the cancer cells.[13] Figure 2 B shows a number of selected peaks that differ between the healthy gastric tissue layers and cancer tissue in the mass range m/z 600–1000 (using Kruskal–Wallis ANOVA).

Colonoscopic procedures involving diathermy are associated with a nine-fold increase in perforation risk compared to a purely diagnostic procedure,[14] and ulcerated lesions undergoing EMR are also at higher risk of perforation.[15] By using the REIMS endoscopic method, it should be possible to incorporate an alert feature in the system so that any diathermy devices are immediately stopped if there is a breach of the submucosal layer during polypectomy or

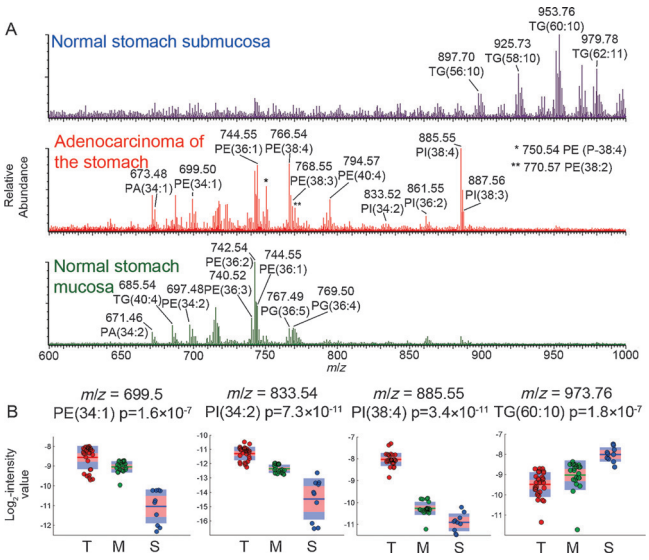

Figure 2. A) Mass spectra from gastric mucosa, gastric submucosa, and adenocarcinoma tissue recorded ex vivo using a modified Xevo G2-S Q-Tof mass spectrometer. Cancerous and healthy mucosal tissues feature mainly phospholipids in the m/z 600–900 region, whilst submucosa features triglyceride and phosphatidylinositol species in the m/z 850–1000 region. B) Comparison of the abundance of selected peaks showing significant differences between cancerous and healthy tissues in the range m/z 600–1000 using Kruskal–Wallis ANOVA, $p < 0.005$. T = tumor, M = mucosa, S = submucosa.

endomucosal resection. Development of the REIMS technology for this purpose could help to decrease perforation rates and the morbidity associated with this complication.

Human colonic adenocarcinoma ($n = 25$, 7 patients), healthy colonic mucosa ($n = 24$, 10 patients), and adenomatous polyps ($n = 13$, 5 patients) were sampled ex vivo and the resulting REIMS data were analyzed using multivariate statistical tools (Figure 3). Spectra were acquired from three diathermy passes directly through the colonic layers and each scan was plotted on a three-dimensional PCA plot (Figure 3C). In agreement with previously published REIMS studies, the spectra acquired from healthy mucosa and adenocarcinoma of both the stomach and colon separate well in three-dimensional PCA space (Figure 3A,B). The sampled adenomatous polyps show diverse classification in the PCA model, in agreement with their precancerous nature and high level of heterogeneity.

Following the analysis of ex vivo samples, the novel REIMS endoscopic method was also tested in vivo on three consecutive patients referred for colonoscopy. Different regions of the colon and rectum were sampled during the colonoscopy procedures. The first and third patients had evidence of colonic polyps, which were confirmed to be benign. The second patient had evidence of a normal colon with no polyps. The mucosal layer showed uniform spectral pattern independently from anatomical location; however colonic polyps showed marked differences from the healthy mucosal layer (Figure 4), in agreement with the observations from the ex vivo studies (Figure 3B).

The data presented in this report demonstrate the significant translational potential for developing the REIMS

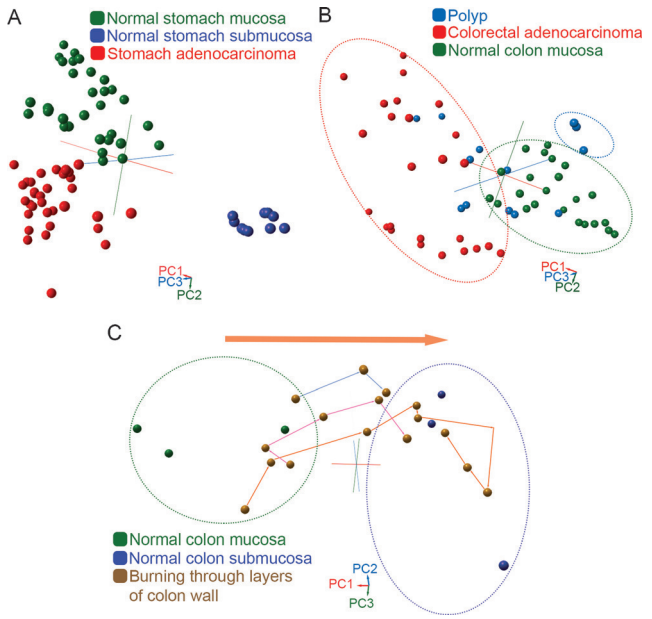

Figure 3. A) Three-dimensional PCA plot for healthy gastric mucosa ($n = 32$), gastric submucosa ($n = 10$), and adenocarcinoma of the stomach ($n = 29$) acquired from three patients ex vivo with a Xevo G2-S Q-Tof mass spectrometer. The differences between submucosa and the other two layers could potentially be used to develop a perforation risk-alert system for interventional endoscopy. B) Three-dimensional PCA plot for human colon adenocarcinoma ($n = 25$, from seven patients) acquired from ex vivo samples, healthy colonic mucosa ($n = 24$, from ten patients), and polyps ($n = 13$ collected from five patients) acquired from ex vivo endoscopy biopsies. The leave-one-patient-out cross-validation of the multivariate model containing spectra obtained by the analysis of normal and cancerous tissue resulted in 95% specificity and 88.5% sensitivity. The ellipsoids represent the polyps grouped with normal mucosa samples (green), cancerous samples (red,) or separately (blue). C) Three-dimensional PCA plot containing 3×3 normal, mucosal and submucosal sampling points and each scan acquired from three diathermy passes through the colonic layers ex vivo starting on the mucosal side (two patients). The arrow represents the direction of the diathermy pass and the consecutive scans are linked together with each burn. There is a clear shift observed from mucosa to submucosa.

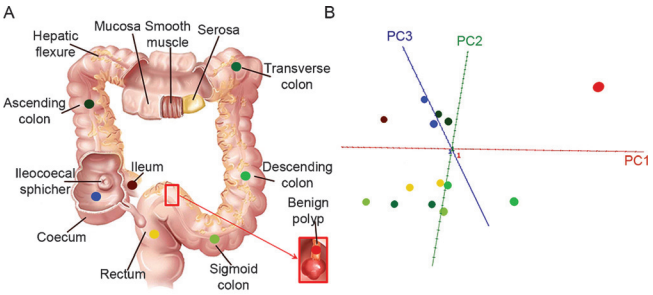

Figure 4. In vivo utilization of the novel REIMS endoscope system. A) Sampling points for three patients undergoing colonoscopy. B) Sampling points depicted on a three-dimensional PCA plot. The spectra acquired in vivo when the polyps were removed localize in a different part of space, whilst all other mucosal spectra are quasi-uniformly independent of the sampling location within the colorectal tract and the ileum.

technique as a real-time diagnostic tool in endoscopy. We developed a novel REIMS setup that is compatible with endoscopic applications and tested the method in both ex vivo and in vivo settings without the need for major modification of standard approved clinical endoscopic equipment. The novel endoscope setup has successfully been shown to be capable of differentiating between healthy mucosa, adenoma, and colorectal cancer based on their individual lipodomic spectral profiles and can therefore be used for the assessment of endoscopic resection margins. Furthermore, the differences demonstrated between mucosal and submucosal layers of the GI tract indicate that REIMS could also be utilized to avoid damaging healthy tissue during interventional endoscopy. REIMS technology has also been demonstrated to be applicable to the identification of microorganisms,[16] which raises the possibility of developing an endoscopic in situ bacterial community analysis tool by using this platform. This novel endoscopic method can also be envisioned as a general approach to be used in course of gastrointestinal screening programs for the early detection of precancerous lesions.

Experimental Section

A commercially available polypectomy snare (Olympus Model No. SD-210U-15, working length 2300 mm, minimum channel size 2.8 mm, opening diameter 15 mm, wire thickness 0.47 mm) was equipped with an additional T-piece in order to establish connection with a 1/8′ OD 2 mm PFTE tubing between the tissue evaporation point and the atmospheric inlet of a mass spectrometer (Xevo G2-S Q-TOF, Waters, Manchester, UK, and LTQ Velos linear iontrap mass spectrometer, Thermo Fisher Scientific ltd, San Jose, CA, USA). The snare was used with a commercially available endoscope (Olympus GIF-H260Z, Olympus Corporation, Tokyo, Japan) and endoscopic stack, coupled with an electrosurgical generator (Valleylab Surgistat II, Covidien, Mansfield, MA, USA). The electrosurgical aerosol plume generated during the removal of polyps was captured through the fenestrations of the snare and transferred to the mass spectrometer through the snare tubing via PFTE tubing coupled directly to the inlet capillary using the internal vacuum of the mass spectrometer for aspiration. High-resolution mass spectrometry was performed in negative-ion mode between m/z 150–1500 range. Written, informed consent was obtained from all patients who provided tissue samples. Ethical approval was obtained from the Hungarian National Scientific Research Ethical Committee (Ref number 182/PI/10) and the National Research Ethics Service, UK (Ref number: 11/LO/0686). The data analysis workflow for the separation of healthy, cancerous, and adenomatous polyps of the gastrointestinal tract includes the construction of a tissue-specific spectral database followed by multivariate classification and spectral identification algorithms as previously described.[11,12]

Acknowledgements

The project was funded by European Union Sevent Framework Programme (FP7/2007–2013 under Grant Agreement No. 297499/305940) and European Research Council Consolidator Grant, Grant Agreement No. 617896 and the National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London.

Keywords: cancer · endoscopy · in vivo characterization · mass spectrometry · phospholipids

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 11059–11062
Angew. Chem. **2015**, *127*, 11211–11214

[1] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, D. Forman, *Ca-Cancer J. Clin.* **2011**, *61*, 69–90.
[2] World Health Organization, The World Health Organization's fight against cancer: strategies that prevent, cure and care, World Health Organization, Geneva, **2007**.
[3] A. Shaukat, S. J. Mongin, M. S. Geisser, F. A. Lederle, J. H. Bond, J. S. Mandel, T. R. Church, *N. Engl. J. Med.* **2013**, *369*, 1106–1114.
[4] C. J. Rees, P. T. Rajasekhar, M. D. Rutter, E. Dekker, *Expert Rev. Gastroenterol. Hepatol.* **2014**, *8*, 29–47.
[5] G. Chadwick, O. Groene, J. Hoare, R. H. Hardwick, S. Riley, T. D. Crosby, G. B. Hanna, D. A. Cromwell, *Endoscopy* **2014**, *46*, 553–560.
[6] M. Zorzi, C. Senore, F. Da Re, A. Barca, L. A. Bonelli, R. Cannizzaro, R. Fasoli, L. Di Furia, E. Di Giulio, P. Mantellini, C. Naldoni, R. Sassatelli, D. Rex, C. Hassan, M. Zappa, *Gut,* DOI: 10.1136/gutjnl-2014-307954.
[7] R. J. Haidry, M. A. Butt, J. M. Dunn, A. Gupta, G. Lipman, H. L. Smart, P. Bhandari, L. Smith, R. Willert, G. Fullarton, M. Di Pietro, C. Gordon, I. Penman, H. Barr, P. Patel, N. Kapoor, J. Hoare, R. Narayanasamy, Y. Ang, A. Veitch, K. Ragunath, M. Novelli, L. B. Lovat, *Gut* **2015**, *64*, 1192–1199.
[8] K. T. Kao, A. Q. Giap, M. A. Abbas, *Arch. Surg.* **2011**, *146*, 690–696.
[9] a) ASGE Technology Committee, *Gastrointest. Endosc.* **2013**, *78*, 568–573; b) X. Qi, Y. Pan, M. V. Sivak, J. E. Willis, G. Isenberg, A. M. Rollins, *Biomed. Opt. Express* **2010**, *1*, 825–847.
[10] a) C. H. Chen, Z. Lin, S. Garimella, L. Zheng, R. Shi, R. G. Cooks, Z. Ouyang, *Anal. Chem.* **2013**, *85*, 11843–11850; b) M. K. Mandal, K. Yoshimura, L. C. Chen, Z. Yu, T. Nakazawa, R. Katoh, H. Fujii, S. Takeda, H. Nonami, K. Hiraoka, *J. Am. Soc. Mass Spectrom.* **2012**, *23*, 2043–2047.
[11] J. Balog, L. Sasi-Szabo, J. Kinross, M. R. Lewis, L. J. Muirhead, K. Veselkov, R. Mirnezami, B. Dezso, L. Damjanovich, A. Darzi, J. K. Nicholson, Z. Takats, *Sci. Transl. Med.* **2013**, *5*, 194ra193.
[12] J. Balog, T. Szaniszlo, K. C. Schaefer, J. Denes, A. Lopata, L. Godorhazy, D. Szalay, L. Balogh, L. Sasi-Szabo, M. Toth, Z. Takats, *Anal. Chem.* **2010**, *82*, 7343–7350.
[13] I. J. Lodhi, C. F. Semenkovich, *Cell Metab.* **2014**, *19*, 380–392.
[14] T. R. Levin, W. Zhao, C. Conell, L. C. Seeff, D. L. Manninen, J. A. Shapiro, J. Schulman, *Ann. Intern. Med.* **2006**, *145*, 880–886.
[15] M. Fujishiro, O. Goto, N. Kakushima, S. Kodashima, Y. Muraki, M. Omata, *Dig. Liver Dis.* **2007**, *39*, 566–571.
[16] N. Strittmatter, M. Rebec, E. A. Jones, O. Golf, A. Abdolrasouli, J. Balog, V. Behrends, K. A. Veselkov, Z. Takats, *Anal. Chem.* **2014**, *86*, 6555–6562.

Received: March 25, 2015
Revised: May 20, 2015
Published online: August 6, 2015

