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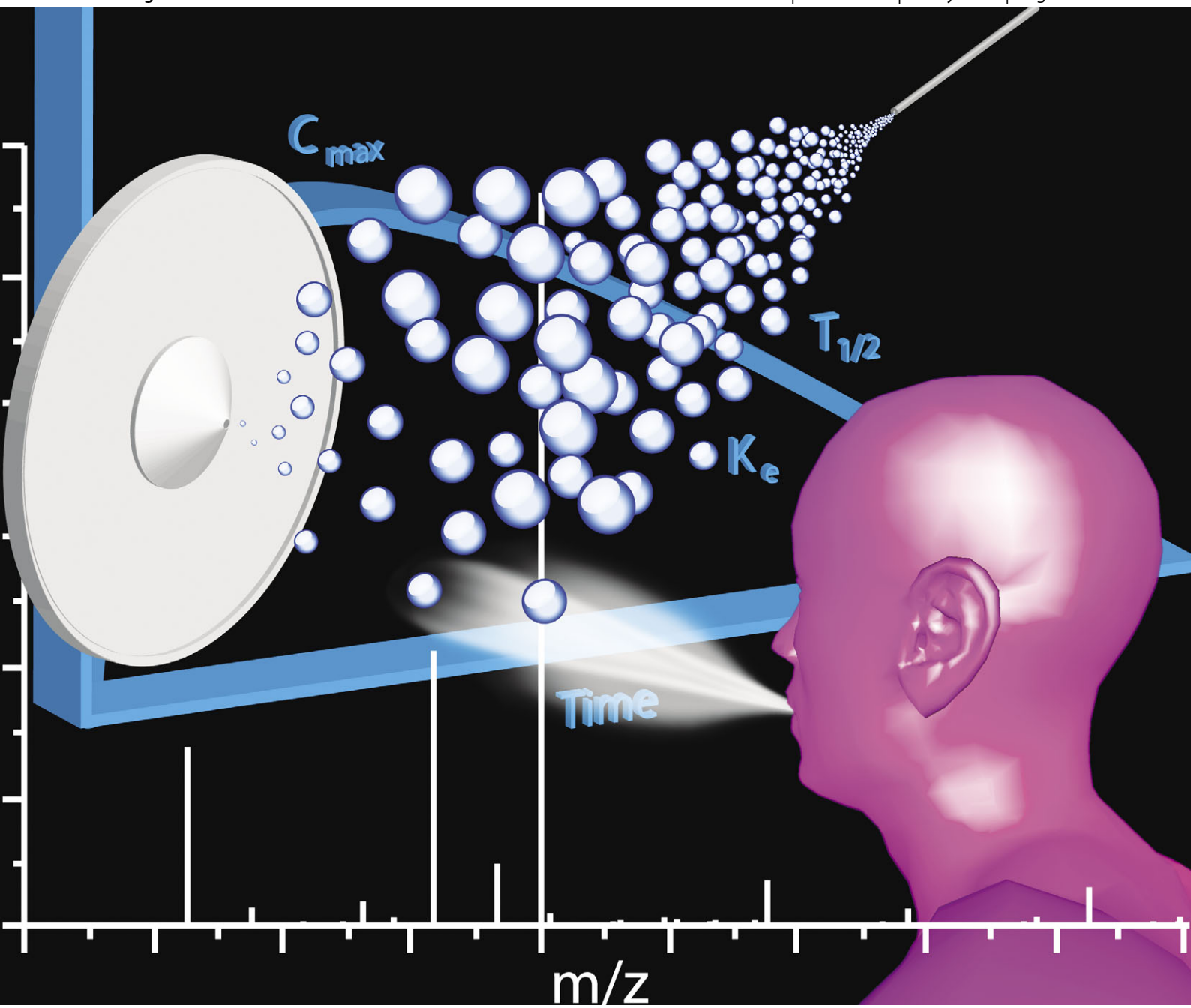

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COMMUNICATION

Real-time, *in vivo* monitoring and pharmacokinetics of valproic acid *via* a novel biomarker in exhaled breath†

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Extractive electrospray ionization mass spectrometry is shown to allow real-time, *in vivo* drug monitoring and pharmacokinetic measurement in a non-invasive, pain-free manner as demonstrated by the mass spectral measurement of a novel exhaled breath biomarker for valproic acid, a medication used to control epilepsy.

The analysis of exhaled breath has received considerable attention[1–5] and is an interesting alternative to blood sampling: provided that a suitable apparatus is available, it does not require specialized personnel or generate hazardous waste. Although its chemical composition is chemically diverse, breath is still a simpler matrix than plasma. In spite of these advantages only a few techniques based on breath sampling have found a niche in clinical or forensic analysis, *e.g.*, to diagnose *H. pylori* infection[3] or the degree of alcohol intoxication.[4] Difficulties encountered in breath analysis include lack of simple or suitable separation, identification, quantitation, and standardization techniques[5]; tedious sample collection and pre-treatment protocols are necessary for some techniques, *e.g.* exhaled breath condensate analysis.[6] On the other hand, a couple of direct breath analysis techniques have been developed, selected ion flow tube (SIFT)[7] and proton transfer reaction (PTR)[8,9] mass spectrometries, but these necessitate instrumentation not widely available and are generally limited to volatile compounds. Recently, extractive electrospray ionization (EESI) mass spectrometry[10] has been applied for real-time analysis of volatiles and non-volatiles in breath[11] thus obviating sample storage and pre-treatment.

Valproic acid (VPA, 2-propylpentanoic acid, MW 144.21 g mol^{-1}) has long been used for treating epilepsy and other neuropsychiatric disorders due to its modulation of the gamma amino butyric acid pathways and/or the sodium/calcium channels.[12,13] The pharmacologically relevant free VPA fraction is influenced by many factors, primarily VPA's protein binding capacity which depends both on the concentration of VPA as well as on the serum albumin concentration[14] (which can be reduced due to different illnesses). In addition, other strongly protein-bound over-the-counter medications may displace VPA from its protein binding site leading to an increase of the VPA free fraction, and even to a change of the metabolic pathways of VPA.

These dependencies necessitate frequent therapeutic drug monitoring as a guide for dosage adjustment, prevention of side effects (see ESI†), and drug compliance. For determining the optimum therapeutic window for VPA, new patients typically give blood every few weeks initially.

We report herein the use of EESI-MS (positive ion mode) for monitoring the intake and clearance of VPA through the analysis of breath with a minimally modified ESI source of a commercial quadrupole time-of-flight mass spectrometer instrument (Fig. 1). This technique allows the real-time determination of pharmacokinetic (PK) profiles and active drug concentration in a simple, non-invasive, and pain-free manner without the need for sample storage or pre-treatment.

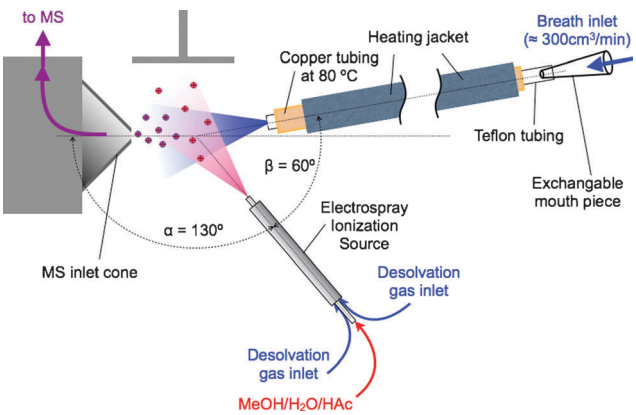

Fig. 1 EESI experimental setup for on-line breath analysis. The breath is introduced through a heated (80 °C) Teflon tube.

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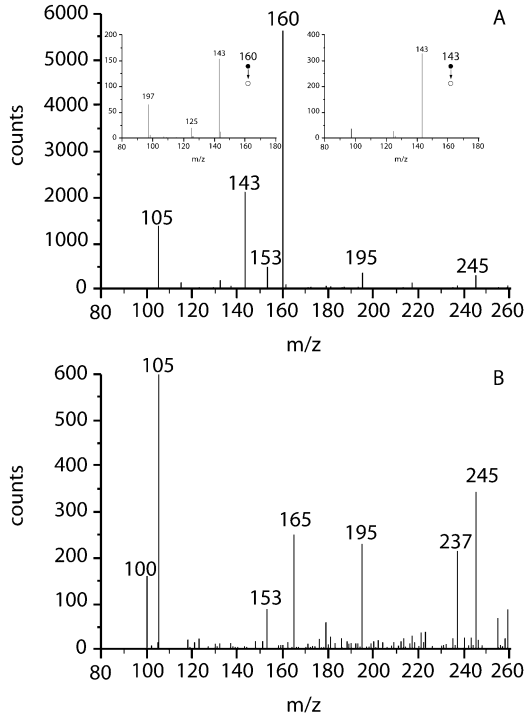

Fig. 2 Breath fingerprints of a volunteer under a daily dose of valproic acid (A) and of a typical healthy volunteer with no valproic acid intake (B). Note the difference in the intensity scale. Insets in (A) show tandem MS fragmentation patterns: the fragment at m/z 143 corresponds to a loss of NH_3, from the peak at m/z = 160; the fragment at m/z 125 corresponds to a subsequent loss of H_2O, and the fragment at m/z 97 corresponds to a subsequent loss of CO. In addition, a fragment at m/z 55 was observed which could correspond to C_4H_7. See text for high resolution MS data.

The breath fingerprint of a volunteer who was under VPA treatment for epilepsy was measured (Fig. 2a, for materials & methods see ESI†). Also, a screening of the breath fingerprint from several healthy volunteers was performed to have a baseline comparison (Fig. 2b). Each spectrum shows distinct peaks, thought to originate from various metabolites present in the breath since EESI MS has been shown to provide a "metabolic fingerprint" of breath.[11] There are, however, striking differences: the breath of the individual under VPA treatment shows major peaks at m/z 143 and m/z 160, as opposed to the breath of the healthy volunteer where peaks at m/z 105, 165, and 245 dominate. Signals at m/z = 143 and 160 started appearing in the breath of healthy volunteers who were administered with a single dose of VPA, proving that these are related to the systemic level of the drug (see ESI†).

We assign the peak at m/z = 160.1330 to a VPA metabolite with the elemental composition $C_8H_{14}O_2$ cationized by an ammonium ion, NH_4^+ (0.5 RDB, Δm = −0.206 ppm). This is supported by accurate mass (<5 ppm) and tandem MS measurements (Fig. 2 insets and ESI†). There are no reported metabolites of VPA with this chemical formula. However, according to a compendium of mass balance studies, the total amount of ingested VPA has not been completely accounted for in humans,[15] *i.e.*, there should still be metabolites that have not been identified. The VPA biomarker observed in breath through EESI MS is suggested to be 4-hydroxy–VPA–γ-lactone (or, less favored, 5-hydroxy–VPA–δ-lactone). All the data obtained support these assignments, for example, the facile fragmentation of m/z 160 to form m/z 143, which is consistent with a noncovalent complex with NH_4^+. This type of complexes have been shown to readily form with lactones,[16] and NH_3 is known to occur in breath.[7] Both 4-OH–VPA–γ-lactone and 5-OH–VPA–δ-lactone standards were synthesized and analyzed *via* EESI, and MS/MS was performed (see ESI†). The fragmentation pattern from the m/z 143 signal was identical for the standard vapors and the exhaled breath. Furthermore, the 4-OH–VPA–γ-lactone standard vapors were mixed with ammonia vapors in the ESI plume and a clear shift to m/z = 160 was observed (see ESI†). Thus, we are confident that the signal at m/z = 143.1065 corresponds to protonated 4-OH–VPA–γ-lactone (or 5-OH–VPA–δ-lactone), $C_8H_{15}O_2$.

The presence of OH–VPA–lactone metabolites in exhaled breath of individuals under VPA therapy has never been reported. VPA can be metabolized *via* cytochrome P450 oxidation reactions (Fig. 3) to give 4-OH–VPA (approx. 5%) and 5-OH–VPA (approx. 4%) (see ESI†). Extraction of VPA metabolites from bodily fluids involves the use of hard acids which inevitably results in lactone formation.[17] However, a fraction of the hydroxy acid metabolites could undergo lactone formation producing a relatively unstrained five or six membered ring (Fig. 3). The resulting 4-OH–VPA–γ-lactone and 5-OH–VPA–δ-lactone have fairly high volatility, facilitating their direct analysis from exhaled breath.

We now show that VPA pharmacokinetics can be followed by monitoring the temporal behavior of the biomarkers in breath spectra. Fig. 4(top) shows the m/z = 143 signal as a function of time for the individual under VPA therapy and for a healthy volunteer after taking a single dose (two tablets). The differences in maximum intensities observed can be explained by the different doses and free VPA concentration for the two individuals. The intensity in the single-dose profile first increases and then decreases exponentially. The time-to-peak is consistent with that previously found in extended release formulations of VPA (5–12 hours).[18] VPA is known to follow first-order elimination kinetics,[18] $C(t) = C_0e^{-kt}$, where C_0 is the initial concentration and k is the elimination rate constant; the half-life is then $t_{1/2} = (\ln 2)/k$. The semi-logarithmic plot in Fig. 4(top) supports first-order clearance kinetics with $t_{1/2}$ = 9.24 h. The PKs of several volunteers were obtained *via* EESI-MS, yielding $t_{1/2}$ values between 7 and 10.5 h (see ESI†),

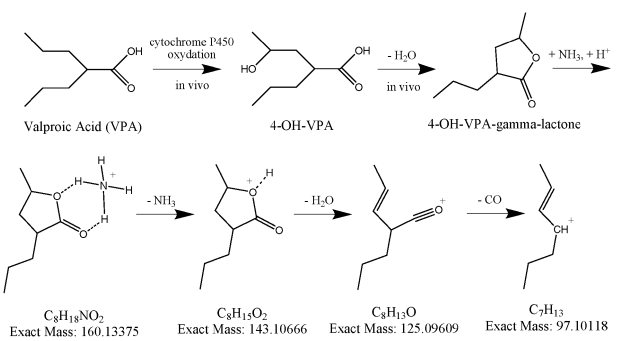

Fig. 3 Valproic acid metabolism pathway to exhaled breath metabolite 4-hydroxy–VPA–γ-lactone (top) and fragmentation pathway of noncovalent ammonium complex of metabolite (bottom).

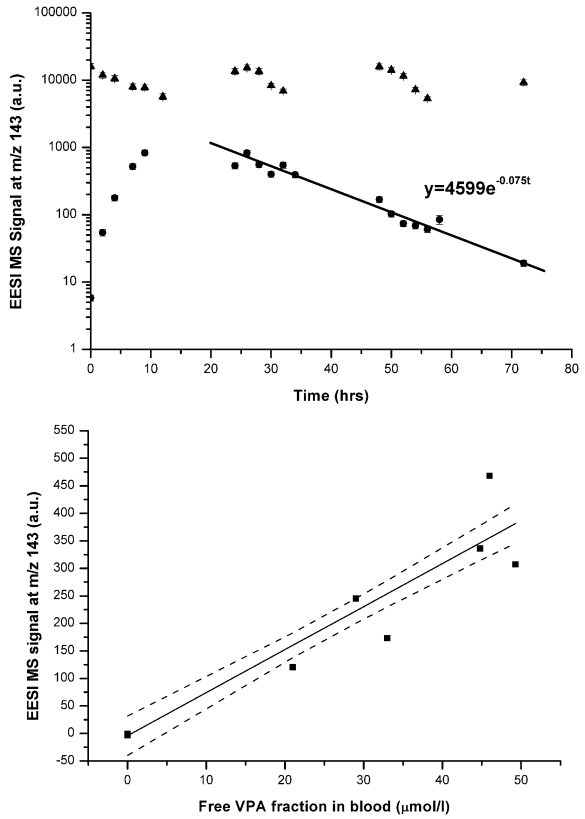

Fig. 4 (top) Signal variation of the exhaled breath VPA marker at $m/z = 143$ as a function of time for volunteer taking a daily dose of four Depakine® Chrono 500 tablets (▲) and volunteer who took a single dose of two tablets (●). In the single dose experiment, 20 hours after ingestion (when the concentration in the body is not increasing anymore), the elimination follows first order kinetics with a half-life of 9.24 hours. (bottom) Correlation of the signal intensity at $m/z = 143$ with the free VPA fraction measured in blood. The dashed lines indicate 95% confidence bands around the linear fit (solid line) with $y = 7.7601x - 1$ ($R^2 = 0.89$).

consistent with the literature for VPA[18] sustained-release formulation. Variations in the constant-dose profile (Fig. 4, top triangles) show that interdose kinetics can be followed.

Finally, we tested whether there was a relationship between the concentration of the VPA metabolite signal in breath and the VPA levels measured in blood. Individuals under VPA medication consistently showed the lactone marker in exhaled breath when analyzed *via* EESI-MS (6 patients, 36 measurements, all male, age 26 to 54, body weights between 57 and 110 kg). The EESI-MS signals for the VPA–lactone in the control group were comparable to the background levels (3 individuals, 18 measurements, all male, age 29 to 48, body weights between 61 and 86 kg). The difference between the two groups is significant ($p \ll 0.01$). Fig. 4(bottom) shows a linear correlation ($R^2 = 0.89$, $p \ll 0.01$) of the EESI signal at $m/z = 143$ plotted against the free VPA fraction in blood, determined by an enzyme-linked immunosorbent assay. This shows that EESI-MS can be used for non-invasive, real-time monitoring of therapeutically relevant VPA levels in patients. Interestingly, a recent study where 3-heptanone was investigated as a potential marker for VPA therapy, using PTR-MS of exhaled breath, found no correlation between 3-heptanone and the weight adjusted VPA daily dose.[9] In the present study, however, even when the EESI MS signal is compared with the weight adjusted VPA daily dose there is a significant correlation ($R^2 = 0.76$, $p \ll 0.01$) albeit less significant compared to the free VPA fraction. The difference may be due to several reasons: (i) 3-heptanone is endogenous in human breath and its concentration may change with certain conditions unrelated to VPA; (ii) the metabolic pathway that forms 3-heptanone is more complex, and involves mitochondrial-oxidation, while hydroxy–VPA forms *via* P450 oxidation in the smooth endoplastic reticulum.

In summary, we have shown that valproic acid pharmacokinetics and ingestion in humans can be monitored through a novel biomarker in exhaled breath. The EESI-MS technique is direct and simple, noninvasive, pain free, and represents an important step toward individualized drug therapy. This study is only a first example of how EESI-MS can be used for following the metabolism of drugs *in vivo*. It has great potential for clinical diagnostics, forensic analysis, as well as metabolomics and personalized medicine.

Notes and references

1 G. Peng, U. Tisch, O. Adams, M. Hakim, N. Shehada, Y. Y. Broza, S. Billan, R. Abdah-Bortnyak, A. Kuten and H. Haick, *Nat. Nanotechnol.*, 2009, **4**, 669–673.
2 D. Smith and P. Spanel, *Analyst*, 2007, **132**, 390–396.
3 B. E. Dunn, H. Cohen and M. J. Blaser, *Clin. Microbiol. Rev.*, 1997, **10**, 720–741.
4 T. A. Brettell and R. Saferstein, *Anal. Chem.*, 1997, **69**, R123–R143.
5 B. Buszewski, M. Kesy, T. Ligor and A. Amann, *Biomed. Chromatogr.*, 2007, **21**, 553–566.
6 P. Montuschi, *Clin. Chim. Acta*, 2005, **356**, 22–34.
7 C. Turner, P. Spanel and D. Smith, *Physiol. Meas.*, 2006, **27**, 321–337.
8 P. Lirk, F. Bodrogi and J. Rieder, *Int. J. Mass Spectrom.*, 2004, **239**, 221–226.
9 S. Erhart, A. Amann, E. Haberlandt, G. Edlinger, A. Schmid, W. Filipiak, K. Schwarz, P. Mochalski, K. Rostasy, D. Karall and S. Scholl-Buergi, *J. Breath Res.*, 2009, **3**, 016004.
10 H. W. Chen, A. Venter and R. G. Cooks, *Chem. Commun.*, 2006, 2042–2044.
11 H. W. Chen, A. Wortmann, W. H. Zhang and R. Zenobi, *Angew. Chem., Int. Ed.*, 2007, **46**, 580–583.
12 *Valproinsäure. Pharmacologie, Klinischer Einsatz, Nebenwirkungen und Therapierichtlinien.*, ed. G. Krämer and J. Walden, Springer-Verlag, Berlin, 2002.
13 D. C. Lagace, W. T. O'Brien, N. Gurvich, M. W. Nachtigal and P. S. Klein, *Clin. Neurosci. Res.*, 2004, **4**, 215–225.
14 A. Dasgupta, *Clin. Chim. Acta*, 2007, **377**, 1–13.
15 V. S. Gopaul, W. Tang, K. Farrell and F. S. Abbott, *Drug Metab. Dispos.*, 2003, **31**, 114–121.
16 M. Stefansson, P. J. R. Sjoberg and K. E. Markides, *Anal. Chem.*, 1996, **68**, 1792–1797.
17 H. Schäfer and R. Lührs, *Arzneim.-Forsch.*, 1978, **28**, 657–662.
18 F. Bressolle, R. Gomeni, R. Alric, M. J. Royermorrot and J. Necciari, *J. Pharm. Sci.*, 1994, **83**, 1461–1464.

