RAPID AND NON-INVASIVE METHOD TO DETERMINE TOXIC LEVELS OF ALCOHOLS AND γ-HYDROXYLBUTYRIC ACID IN SALIVA SAMPLES BY GAS CHROMATOGRAPHY-DIFFERENTIAL MOBILITY SPECTROMETRY

[+]L. Criado-García[(1)], [+]D.M. Ruszkiewicz[(2)], G.A. Eiceman[(2)], CLP. Thomas[(2)]*

(1) Department of Analytical Chemistry. Annex C-3 Building. Campus of Rabanales. Institute of Fine Chemistry and Nanochemistry. University of Cordoba. 14071 Córdoba, Spain.

(2) Centre for Analytical Science, Department of Chemistry, Loughborough University. Loughborough, LE11 3TU, UK.

*E-mail: C.L.P.Thomas@lboro.ac.uk

Keywords: methanol, ethanol, ethylene glycol, 1,3-propandiol , γ-hydroxybutyric acid, saliva, thermal desorption gas chromatography differential mobility spectrometry.

Abstract

A polydimethylsilicone oral sampler was used to extract methanol, ethanol, ethylene glycol, 1,3-propandiol and γ-hydroxybutyric acid from samples of human saliva obtained using a passive-drool approach. The extracted compounds were recovered by thermal desorption, isolated by gas chromatography and detected with differential mobility spectrometry, operating with a programmed dispersion field.

Complex signal behaviours were also observed that were consistent with hitherto unobserved fragmentation behaviours in differential mobility spectrometry. These yielded high-mobility fragments obscured within the envelope of the water-based reactant ion peak. Further, compensation field maxima shifts were also observed attributable to transport gas modification phenomena. Nevertheless, the responses obtained indicated that in-vivo saliva sampling with thermal desorption gas chromatography may be used to provide a semi-quantitative diagnostic screen over the toxicity threshold concentration ranges of 100 mg.dm^{-3} to 3 g.dm^{-3}. A candidate method suitable for use in low resource settings for the non-invasive screening of patients intoxicated by alcohols and volatile sedatives has been demonstrated.

[+] L. Criado-García and D.M. Ruszkiewicz should be considered as joint first authors.

Introduction

Treatment of poisoning from the consumption of ethanol and the management of the intoxicated patient represents a significant burden on many health services. Further, poisoning from the consumption of other alcohols notably methanol and ethylene glycol occurs sporadically and from time-to-time outbreaks occur where food, drink or medicines are contaminated, resulting in significant mortality [1]. The essence of diagnosis is speed, and even in well–resourced health-care settings the collection of blood samples for remote analysis may introduce delays that prevent effective treatment. In communities and regions without recourse to gold-standard pathology services, or in cases where the numbers of patients runs into the hundreds [2] clinical teams may be forced to treat their patients symptomatically without recourse to reliable diagnosis. Other complications arise when intoxication from sedatives, such as γ-hydroxylbutyric acid (GHB) taken intentionally or through malicious administration, is mistaken for ethanol abuse, or more seriously is masked by ethanol consumption. Consequently it would appear there is a need, across all scales of health services, for faster point-of-care toxicity screening for methanol, ethanol, ethylene-glycol and 1,3-propandiol. It would also be helpful to be able to screen simultaneously for the presence of sedatives such GHB, and more recently, γ-butyrolactone.

Ethylene glycol and 1,3-propandiol are highly soluble in aqueous media with Henry's Law constants of $4x10^{6}$ $mol.kg^{-1}.bar^{-1}$ and 910,000 $mol.kg^{-1}.bar^{-1}$ respectively making headspace analysis and, by implication, exhaled breath analysis unlikely to be practicable; note that in comparison the Henry's Law constants for methanol and ethanol are 230 $mol.kg^{-1}.bar^{-1}$ and 190 $mol.kg^{-1}.bar^{-1}$ respectively.

The utility of using saliva analysis for profiling methanol intoxication was proposed in 2009 [3] and subsequently an active membrane [4 and 5] was used to recover and analyse methanol and ethanol from human saliva with determination by thermal desorption gas chromatography differential mobility spectrometry (TD-GC-DMS) across the concentration range 30 $mg.dm^{-3}$ to 500 $mg.dm^{-3}$ [6]. At that time the possible utility of extending the approach to glycols was noted. In saliva fluids, drugs of abuse have been reported to be detectable for between 5 and 48 hr in the $ng.cm^{-3}$ range [7] supporting the proposition of the development of saliva based screens. Indeed, methanol and ethanol present in saliva samples has been

previously determined by GC-FID, with the proposition for extending the approach from confirmatory analyses to routine application in toxicology laboratories[8].

This research focussed on methanol, ethanol, ethylene glycol, 1,3-propandiol and GHB. Ethanol toxicity is dependent on individual tolerance and use, although levels greater than 3 $g.dm^{-3}$ to 4 $g.dm^{-3}$ may be fatal due to respiratory depression and blood ethanol concentrations between 500 $mg.dm^{-3}$ and 700 $mg.dm^{-3}$ may be considered to be the highest that may be tolerated without neurological effects [9]. Methanol, may cause metabolic acidosis, neurologic injuries, and death when ingested and blood-serum methanol levels greater than 200 $mg.dm^{-3}$ correlate with ocular injury, while the minimal lethal dose of methanol in adults is believed to be 340 $mg\ kg^{-1}$ of body weight [10]. Ethylene glycol is moderately toxic and its toxic by-products first affect the central nervous system, then the heart, and finally the kidneys. Current recommendations are that treatment with Fomepizole is initiated immediately if serum concentrations of methanol or ethylene glycol exceed 200 $mg.dm^{-3}$. In contrast ingestion of 1,3-propandiol is not as serious and large quantities are required to cause perceptible health damage in humans with blood-plasma concentrations over 4 $g.dm^{-3}$ associated with serious harm [11]. GHB has useful therapeutic uses such as treating narcolepsy, however it is also a drug of abuse and is associated with assault. The therapeutic range is narrow and accidental overdosing is a common cause of injury with potentially fatal outcomes, normally associated with cardiorespiratory arrest [12].

The current work sought to extend the earlier TD-GC-DMS study to include a semi-quantitative diagnostic screen of alcohol toxicants and GHB based on a non-invasive saliva sampling methodology and establish if direct extraction from saliva to a polydimethylsilicone coupon [13] with recovery and analysis by TD-GC-DMS was feasible. The DMS platform was chosen as this technique has been demonstrated to be effective for the rapid, robust and sensitive detection and quantitation of alcohols in low resource settings [14].

Experimental

Ethics, participant preparation and saliva sampling.

It is helpful to note at the outset that the volunteers who participated in this research were not exposed to any chemical hazard(s). The study was conducted in

accordance with the ethical principles of Good Clinical Practice and the Declaration of Helsinki. The local ethics committee (Ethical Advisory Committee, Loughborough University, Loughborough, LE11 2DT) approved the studies (References G10-P23 and G10-P24) Three healthy adult male non-smokers volunteered to participate in this study and gave written informed consent. The participants were recruited from Loughborough University staff, students and their social networks. Each participant provided two samples throughout the experimental campaign.

On the morning of their study visit the participant were asked not to: brush their teeth; use any personal care products, or eat breakfast. Participants were also asked to only drink cold water, and refrain from flavoured, caffeinated, or drinks containing fruit juice(s). All saliva samples were taken in an in-vivo sample station located in a small internal room, where privacy was ensured, at the Centre for Analytical Science at the Chemistry Department of Loughborough University. A chaperone, of the same gender as the participant, was present during sample collection and access was restricted to only those researchers and participants involved in the sampling process. After an introduction to the study the participants were familiarised with the passive drool approach that was used to obtain a sample of their saliva [13], before proceeding to provide approximately 10 cm^3 of saliva. The participants sat with their head tilted forward to cause saliva to pool at the front of their mouth and then drain from their lips into a glass collection vial. On completion of sampling the vial was sealed promptly with a Teflon™ faced screw-top cap. Immediately after sampling the saliva was transferred to the laboratory where 1.8 cm^3 aliquots of the saliva sample was pipetted into 2 cm^3 chromatography vials, which were sealed immediately with a screw cap fitted with a silicone septum. These saliva aliquots were used immediately, within 3 hr of collection and maintained at ambient temperature (20°C ± 2°C) until disposal. Saliva residues were disposed immediately after use by diluting with a disinfectant solution and rinsed down a sink with a copious flow of running water. No cells or DNA were retained or stored.

Chemicals

Ethanol, methanol, ethylene glycol, 1,3-propandiol , sodium chloride (purity of these compounds ≥99.8%) and butanoic acid, 4-hydroxy-, ammonium salt (GHB) in methanol (1 $mg.cm^{-3}$) were obtained from Sigma Aldrich; see Table 1. He carrier gas

was obtained from BOC, UK, and purified by passing through two triple-bed gas purifiers mounted in series (Thames Restek). Nitrogen was generated on site (PEAK Scientific, UK, model nk-10L-HP) and purified by passing through a charcoal adsorbent-bed gas-purifier (Varian), a moisture filter (Varian), and a triple-bed gas purifier (Thames Restek), all mounted in series. Water (>18MΩ) was generated on site.

PDMS saliva sampler.

A titanium cylinder (6 mm long, 2 mm o.d. C-SPTD5-6MM Markes International Ltd) coated on the internal and external surfaces with polydimethylsiloxane (internal wall thickness 1 μm and external wall thickness 0.5 mm). was used to recover VOCs from the saliva. This approach has been described previously for the in-vivo sampling of Saliva VOCs [13]. The saliva sampler was prepared by cleaning with Milton® sterilising liquid (Suffolk, UK) and then rinsing with deionised water before conditioning under vacuum at 190°C for 15 hr. Once conditioned the PDMS rods were inserted into a cleaned and conditioned glass thermal desorption tube and thermally desorbed for 10 min at 190 °C; the resultant GC-MS trace provided verification that the PDMS sampling media was free of contamination. On removal from the thermal desorption unit the thermal desorption tube containing the PDMS coated titanium cylinder was immediately capped, sealed and stored at 4 °C. Before use the saliva-samplers were thermally desorbed again under the conditions in Table 3 to remove any traces of possible VOC contamination that may have occurred during storage and to provide further verification that the sampler was free of contamination.

Instrumentation

Two instrument configurations were used in this study. Method development and calibration were undertaken using liquid injections to a GC-DMS. Characterisation of the recovery of the analytes from spiked saliva samples was undertaken using a thermal desorption unit interfaced to the GC-MS (Figure 1).

Multi-linear regression was used to optimise the differential mobility spectrometer operating parameters of: dispersion-field; temperature; number of compensation-field steps; and compensation-field step duration (DOE PRO XL Software for Microsoft Excel, SigmaZone). Data were generated from a central composite design

(CCD) with each of the 4 factors at 4 levels with replicates at five different concentrations, see Table 2 [**15].** The DMS parameters were optimised for maximum sensitivity while maintaining "satisfactory" resolution between the ion clusters generated within the ^{63}Ni ionisation source see Table 3, and Figures 2, 3 and 4.

Calibration of the DMS under optimised conditions was undertaken using gas chromatography to introduce known on-column masses of the analytes injected in a solution of dichloromethane, Figure 1 and Table 3. A 30 m long wall-coated open-tubular capillary GC column with an internal diameter of 0.32 mm and a 0.5 µm thick trifluoropropylmethylpolysiloxane stationary phase (Rtx-200MS, Restek, UK) was interfaced to a DMS with a heated transfer-line configured as a sheath flow interface [16] made from a 20 cm length of ¼" stainless steel tubing with the GC-column axially aligned within the tube. The transfer-line was heat-traced with heating tape and maintained at 100°C.

The DMS used in the study was a planar device (SDP-1 Sionex, MS USA) with a 0.5 mm gap between the two parallel electrodes and a 5.9 MBq ^{63}Ni ionisation source. The DMS was controlled through a virtual instrument (Sionex DMx Expert, Version 2.4.0) run on the Dell laptop (Inspiron, 4000) The data were saved to a Microsoft Excel spreadsheet file for post-run processing. The transport gas was purified nitrogen with water concentrations maintained in the range 22.5 to 26.3 mg.m^{-3} (Panametrics Series 35 hygrometer)

A two-stage thermal desorption unit (Markes International Unity 2,) was used to recover VOC extracts from saliva samples with a cold trap for refocusing the recovered VOCs packed with a mixed bed of Tenax TA and Carbograph 1TD. The 1.5 m long transfer line to the GC-column was a deactivated methyl-capped capillary column (Restek, UK) with an internal diameter of 0.23 mm i.d. maintained at 110°C.

Characterisation of spiked saliva samples

The concentration ranges used in this study are summarised in Table 4. A 100 mg.cm^{-3} aqueous stock solution of ethanol, methanol, ethylene glycol, and 1,3-propandiolwas prepared and aliquots of the volumes required to generate the required concentrations were spiked into the saliva samples within three hours of

the saliva being collected. To account for the lower concentration of the GHB standard, and to maintain a constant saliva background in the GHB characterisation experiments a different approach was adopted. Here 0.9 cm^3 of the saliva was used, and spiked with the required aliquot volume of the 1 mg cm^{-3} GHB methanolic solution, before the volume was made up to 1.8 cm^3 with physiological saline ($NaCl_{(aq)}$ 8.5 $g.dm^{-3}$). The ammonia present in the saliva and the GHB salt co-eluted with methanol and suppressed the formation of methanolic product ions (Ammonia has a higher proton affinity than methanol). This interference was eliminated by the addition of 150 µl of 8 % HCL solution into the saliva samples before the sampling rod was placed into the vial.

Once the analytes had been added the saliva standards were homogenised. Immediately this had been done a PDMS coated titanium cartridge was removed from its sealed thermal desorption tube and placed into the vial, which was then sealed immediately. It was important that this procedure was undertaken in a fast and reproducible manner to minimise the effects of evaporative losses in the study. The sealed vial was then placed into a heating-block, maintained at 37 °C for 10 minutes. At the end of the extraction-time the vial was uncapped and the PDMS coated titanium cartridge was removed with stainless steel tweezers and excess fluid removed by gently wiping it with a lint-free wipe ('Kimcare' Kimberly-Clark Professional, UK). The PDMS coated titanium cartridge was then placed immediately into its glass thermal desorption tube and analysed. Cross-contamination checks were run by taking blank runs between every measurement.

Results and discussion

Evaluation of responses

Figure 2 shows the GC-DMS response surfaces from methanol (A), ethanol (B), ethylene glycol (C), 1,3-propandiol(D) and GHB (E) at three levels of column-loading that span the ranges of analyte concentrations associated with the physiological thresholds of these compounds. The four dispersion-field levels used (Table 3) enabled analytical responses to be resolved

Figure 3 shows background subtracted differential mobility spectra obtained from the responses shown in Figure 2 for methanol (A), ethanol (B) and ethylene glycol (C), while Figure 4 compares the responses for 1,3-propandiol(D) and GHB (E). The

dotted lines in these figures indicate the boundary of the reactant ion peak that was removed by the background subtraction in the data processing. The observed responses were complicated with shifts in compensation-field maxima with increasing concentration, and the generation of features embedded within the reactant ion peak that were only discernible after background subtraction. Such phenomena were indicative of the formation of fragment ions and, or, "auto-modification" of the alpha functions of the product ion.

With a dispersion-field of 25 kV.cm-1 and a column-loading of 10.2 ng, methanol yielded a single peak attributed to a hydrated protonated monomer cluster ion at a compensation-field of -444 V.cm-1, that was partially obscured within the reactant ion peak. Reducing the column-loading to 2.0 ng resulted in a compensation-field shift for the hydrated protonated monomer cluster ion to -439.6 V.cm-1 At a lower limit of a 0.20 ng column-loading, only one peak was observed at -439.6 V.cm-1. These observations are consistent with an increase in the alpha-function [17] of a hydrated protonated methanol cluster ion with increasing methanol concentration [18] (modification of the transport gas.At a dispersion-field of (18 kV cm^{-1}) ethanol also yielded a complicated response with features consistent with the generation of fragment ions overlaid with unresolved hydrated protonated monomer cluster ions, and hydrated proton-bound cluster ions, observed to fall at approximately -154.2 $V.cm^{-1}$ for the hydrated protonated monomer cluster-ion and -122.8 $V.cm^{-1}$ for the proton-bound dimer cluster ion. Two features attributed to fragment ions were observed to be obscured within the reactant ion peak. One was at a compensation-field of -238 $V.cm^{-1}$ at a column-loading 40.7 ng and -230 $V.cm^{-1}$ at column-loadings of 15.3 ng and 5.1 ng. The other was present at -216.6 $V.cm^{-1}$ at 40.7 ng, shifting to -207.6$V.cm^{-1}$ at 15.3 ng and 5.1 ng.

At a dispersion-field of 22 $kV.cm^{-1}$ ethylene glycol produced a clearly resolved feature at -51.6 $V.cm^{-1}$, attributed to a proton-bound dimer cluster ion, along with two features obscured by the reactant ion peak. The weakest of these features, completely obscured by the reactant ion peak was observed at a compensation-field of -403.8 $V.cm^{-1}$ across the range of column-loadings with no indication of auto-modification observed. However, the other feature, partially obscured by the reactant ion peak, was observed to shift from a compensation-field of -359.2 $V.cm^{-1}$, with a column-loading of 81 ng, to -337 $V.cm^{-1}$ with a column loading of 14.3 ng.

The lowest column loading applied generated a low intensity split peak that straddled -337.4 $V.cm^{-1}$. At the same dispersion-field (22 $kV.cm^{-1}$) 1,3-propandiolyielded two features, both resolved from the reactant ion peak. The peak at a compensation-field of 15.4 $V.cm^{-1}$ was attributed to a proton bound dimer ion cluster. The feature attributed to a hydrated monomer-ion cluster was observed to shift to more negative compensation-fields with increasing column-loading. At 80 ng the peak maximum was at a compensation-field of -283.4 $V.cm^{-1}$, shifting to -261.2 $V.cm^{-1}$ when the column-loading was reduced to 2.7 ng.

GHB also showed complex behaviour with a dispersion-field of 23$kV.cm^{-1}$. In addition to well-resolved hydrated protonated monomer cluster ions and proton-bound dimer cluster ions, fragment ions obscured within the RIP envelope were also evident, and the compensation-field maxima of these fragment ions shifted with increasing column-loading of GHB. Hydrated protonated monomer cluster ions had a compensation field peak maximum at –131.8 $V.cm^{-1}$ and the proton-bound dimer compensation-field maxima was observed at 33.2 $V.cm^{-1}$. No discernible trend in a shift in compensation-field maxima was observed with column loading for the hydrated protonated monomer cluster ion, and the proton-bound dimer was not formed at the lowest column loading of 0.51 ng. At a column loading of 12.9 ng two unresolved fragment ions were discernible within the RIP envelope. The most intense feature was at 310.2 $V.cm^{-1}$ with a shoulder at -296.8 $V.cm^{-1}$. Reducing the column loading to 1.9 ng resulted in a single fragment ion with a compensation-field peak maximum at -301.2 $V.cm^{-1}$ and at a column loading of 0.51 ng the fragment ion was still observable with a compensation-field peak maximum of -288 $V.cm^{-1}$.

Despite the complexity of the responses it was possible to generate well resolved and analytically useful chromatographic peaks, see Figure 5. The chromatograms for ethylene glycol, 1,3-propandiol and GHB were generated by integration of the differential mobility spectra across the proton-bound dimer ion features and the hydrated protonated monomer responses, and these are shown as discrete traces in Figure 5 overlaid with the summed chromatographic response. For ethanol and methanol the chromatograms were generated by integration of the complicated features that contained hydrated protonated monomers and proton-bound dimer ions. Figure 5 shows the intensities of the peaks reflecting the differences in the ionisation efficiencies as well as the column-loadings of the five compounds. Of

particular note was the behaviour of ethylene glycol with a peak shape that indicated a saturated response with significantly lower sensitivity compared to the other four compounds.

Calibration

Calibration of the differential mobility spectrometer was based on the peak volumes for the proton-bound dimer ion responses for the two glycols and GHB. The ethanol calibration was based on the integration of the complicated feature containing unresolved hydrated protonated monomers ion clusters and proton-bound dimer ions while the methanol calibration peak volume was taken from the proton-bound monomer ion response. Table 4 summarises the calibration parameters data.

Saliva analysis

The responses obtained from saliva spiked with a range of concentrations of the analytes are summarised in Figure 6 and Figure 7. The chromatography contained substantial numbers of recovered compounds from the saliva sample, nevertheless it was possible to identify the analytes reliably based on their compensation field and retention times.

The intensities of the responses observed reflected the combined interactions of: the adsorption/absorption behaviour of the analytes onto/into the PDMS sampler medium; the product ion dynamics noted above of the five compounds; and interactions with the saliva matrix. Matrix interactions in drooled saliva are problematic in that microbiological activity and stability of the analytes are likely to be related to the analyte's concentration and will have a time dependent element. Further the physical chemical properties of the saliva may also vary between samples. The previous study with this sampler contrasted the responses obtained from drooled saliva samples against those obtained by sampling directly in the mouth, under the tongue next to the salivary glands. Sampling in the mouth was found to be more sensitive and more reproducible than adopting a passive drool approach. Further, obtaining a passive drool sample requires significantly more patient / participant training and compliance than placing a small rod under their tongue and as such is likely to be a more practical approach to working with patients/participants who may have analytes at levels high enough to be a cause of concern for their safety and welfare [13]. Nevertheless the adoption of a passive

drooled-saliva approach enabled a matrix that approximated the intended sampling conditions to be acquired safely and practicably. Finally, the loss of the more volatile methanol and ethanol to the saliva headspace and hence from the experiment also needs to be acknowledged as a methodological weakeness.

The fragmentation behaviour observed during the method development stage with liquid injections was not observed in the saliva studies. The presence of other closely eluting components within the chromatogram made background subtraction problematic and subsequently it was not possible to investigate fragment ion artefacts with confidence. The on-column masses of methanol recovered were estimated to fall in the range 0.35 ng to 3 ng over the range 100 $mg.dm^{-3}$ to 2 $g.dm^{-3}$, and similarly for ethanol the on-column masses were estimated to fall in the range 29 ng to 42ng. Recoveries for ethylene glycol fell were lower with up to 7 ng obtained at high saliva loadings of 3 $g.dm^{-3}$, contrasted with 1,3-propandiol recoveries of up to 22 ng at the same level. GHB was the most efficiently recovered from saliva with 10 ng recovered at 100 $mg.dm^{-3}$ increasing to an estimated on-column mass of 34 ng at 400 $mg.dm^{-3}$.

Conclusion.

This pilot study demonstrates the effective recovery, detection and semi-quantitative estimation of all the analytes of interest to this work. This represents a potentially useful methodological advance in the rapid assessment of alcohol toxicity and embodied within a TD-GC-DMS or a TD-GC-IMS for it provides a fieldable approach for a rapid screen and evaluation protocol for alcohols present at toxic levels from a single non-invasive sample. This has not been possible previously and has the potential for the development of point-of-care toxicity assessment in emergency room settings. Indeed this study, in concert with others, is developing the concept of extending volatile biomarker measurement from breath to a range of excretory routes. There are instances when breath sampling might be problematic (a propensity for an inebriated patient to vomit for instance) and as such skin and saliva offer alternative routes and techniques for studying and exploiting the tissue/blood/breath-skin-saliva excretion mechanics for non-invasive diagnostics [17].

The apparently simplicity of the analytes belies significant complexity in the ion chemistries associated with their detection using ambient ionisation or radioactive ionisation approaches. Earlier mass spectrometric studies with alcohols have identified the formation of fragment ions associated with proton transfer ionisation approaches [18, 19 and 20]. The presence of signals due to product ion fragmentation would not appear to be without precedent. The alcohol product ions, and their fragment ions, are highly mobile and therefore are associated closely with the water-based reactant ion signals. Increasing resolution between the reactant ion signal and analyte signals by increasing the dispersion field strength has the combined effect of reducing the analytical sensitivity by reducing the analytical area of the ion filter while at the same time promoting fragmentation reactions [21 and 22]. The possible ion fragmentation of GHB has not been reported previously.

Compensation field maximum shifts attributable to the auto-modification of the transport gas by analyte neutrals was also observed and this is an area that will require further investigation to characterise it completely.

The study of fragmentation mechanisms, products and their ramifications for alcohol determination by differential mobility spectrometry along with the development of detection and signal processing algorithms to enable peak-shift from auto-modification of the differential mobility transport gas to be handled efficiently are logical next steps in the development of this area. In parallel to such a study will be the refinement of the methodology to reduce the chromatographic run time to less than 300 s, and the continued development of the sampling approach to reduce the sampling time so that a total analytical run time of 600s might be achieved. This would to enable the delivery of a clinical pilot study within an appropriate poisons unit to assess the efficacy of this approach in patients, benchmarked to current gold-standard toxicity screens.

Acknowledgement

The Authors wish to thank the Spanish Ministry of Education, Culture and Sport for the pre-doctoral grant (AP 2009-3528) for the support of L. Criado-García and the Engineering and Physical Science Research Council alongside John Hoggs Technical Solutions for the support of D.M. Ruszkiewicz through an Industrial Case

387 Studentship Award. The authors also acknowledge and thank the volunteers who
388 participated in this research.

389 ## References

1 Zhang G. Crews K. Wiseman H. Bates N. Hovda K.E. Archer J.R.H. and Dargan P. I. 2012 Application to Include Fomepizole on the WHO Model List of Essential Medicines URL: http://www.who.int/selection_medicines/committees/expert/19/applications/Fomepizole_4_2_AC_Ad.pdf, visited 21 September 2015.

2 BBC News 2015 India alcohol poisoning: Mumbai death toll tops 100, URL: http://www.bbc.co.uk/news/world-asia-india-33224514, visited 21 September 2015.

3 Heberlein A. Lenz B. Degner D. Hornhuber J. Hillemacher T. and Bleich S. 2009 Methanol Levels in Saliva—A Non-Invasive Parameter That May Be Useful in Detection of Alcohol Intoxication. *Alcohol and alcoholism* **45** 126-127.

4 Rezgui N.D. Kanu A.B. Waters K.E. Grant B.M.B. Reader A.J. and Thomas C.L.P. 2005 Separation and preconcentration phenomena in internally heated poly(dimethylsilicone) capillaries: preliminary modelling and demonstration studies *Analyst*, **130** 755-762.

5 Kanu A.B and Thomas C.L.P. 2006 The presumptive detection of benzene in water in the presence of phenol with an active membrane-UV ionisation differential mobility spectrometer Analyst, **131**, 990-999.

6 Bocos-Bintintan V. Moll V.H. Flanagan R. J. and Thomas C. L. P. 2010 Rapid determination of alcohols in human saliva by gas chromatography differential mobility spectrometry following selective membrane extraction Int. J. Ion Mob. Spec. **13** 55-63.

7 Verstraete, A. 2004 Detection Times of Drugs of Abuse in Blood, Urine and Oral Fluid. *Ther. Drug. Monit.* **26** 200-205.

8 Mergen G. Kayaalti Z. Dural E. Aliyev V. Kaya S. Yalcin S. Karakus A. and Soylemezoglu T. 2011 Simultaneous Headspace-GC-FID Analysis for Methanol and Ethanol in Blood Saliva and Urine: Validation of Method and Comparison of Specimens *LC GC EUROPE* **24** 292-297.

9 Jones A.W. 1993 Pharmacokinetics of ethanol in Saliva: Comparison with Blood and Breath Alcohol Profiles, Subjective Feelings of Intoxication, and Diminished Performance. *Clin. Chem.* **39**, 1837-1844.

10 Finkelstein Y. and Vardi J. 2002 Progressive parkinsonism in a young experimental physicist following long-term exposure to methanol *Neurotoxicology* **23** 521-525.

11 Flanagan R. J. Braithwaite R. A. Brown S. S. Widdop B. and de Wolff F. A. 1995 The International Programme on Chemical Safety: Basic Analytical Toxicology Publ. World Health Organisation Geneva.

12 Zvosec D.L. Smith S.W. Porrata T. Strobl A.Q. and Dyer J.E. 2011 Case series of 226 γ-hydroxybutyrate-associated deaths: lethal toxicity and trauma *Am. J. Emerg. Med.* **29** 319–332.

13 Martin H. J. Riazanskaia S. and Thomas C. L. P. 2012 Sampling and characterisation of volatile organic compound profiles in human saliva using a polydimethylsiloxane coupon placed within the oral cavity *Analyst* **137** 3627-3634.

14 Limero T. Reese E. Wallace W.T Cheng P. and Trowbridge J. 2012 Results from the air quality monitor (gas chromatograph-differential mobility spectrometer) experiment on board the international space station Int. J. Ion Mobil. Spec. **15** 189–198.

15 Ali Awan M. Fleet I. and Thomas C. L. P. 2008 Optimising cell-temperature and dispersion-field strength for the screening for putrescine and cadaverine with thermal desorption gas chromatography differential mobility spectrometry *Anal. Chim. Acta* **611** 226-232.

16 Young D. Thomas C. L. P. Breech J. Brittain A. H. and Eiceman G. A. 1999 Extending the concentration and linear dynamic range of ion mobility spectrometry with a sheath flow inlet *Anal. Chim. Acta*, **381** 69-83.

17 Amann A. (Editor) Smith D. (Editor) 2013 Volatile Biomarkers: Non-Invasive Diagnosis in Physiology and Medicine Publ. Publ. Elsevier ISBN 978-0444626134.

18 Friedman L. Long F.A. and Wolfsberg M. 1957 Study of Mass Spectra of Lower Aliphatic Alcohols *J. Phys. Chem.* **27** 613–622.

19 Smith S.C. McEwan M.J. Giles K. Smith D. Adams N.G. 1990 Unimolecular decomposition of a polyatomic ion a variable temperature selected ion flow drift tube: experiment and theoretical interpretation. *Int. J. Mass Spectrom. and Ion Process.* **96** 77-96.

20 Karpas z. Eiceman G.A. Ewing R.G. Harden C.S. 1994 Collision induced dissociation studies of protonated alcohol and alcohol-water clusters by atmospheric pressure ionization tandem mass spectrometry. Part 2. Ethanol, propanol and butanol *Int. J. Mass Spectrom. and Ion Process.* **133** 47-58.

21 Eiceman G.A. Karpas Z. Hill H.H. 2013 Ion mobility spectrometry 3rd Ed. Publ. CRC Press ISBN 9781439859971.

22 Shvartsburg A. 2008 Differential Ion Mobility Spectrometry Publ. CRC Press ISBN 9781420051063

RAPID AND NON-INVASIVE METHOD TO DETERMINE TOXIC LEVELS OF ALCOHOLS AND γ-HYDROXYLBUTYRIC ACID IN SALIVA SAMPLES BY GAS CHROMATOGRAPHY-DIFFERENTIAL MOBILITY SPECTROMETRY
TABLES

L. Criado-García[(1)], D.M. Ruszkiewicz[(2)], G. A. Eiceman[(2)], CLP. Thomas[(2)]*

(1) Department of Analytical Chemistry. Annex C-3 Building. Campus of Rabanales. Institute of Fine Chemistry and Nanochemistry. University of Cordoba. 14071 Córdoba, Spain.

(2) Centre for Analytical Science, Department of Chemistry, Loughborough University. Loughborough, LE11 3TU, UK. *E-mail: C.L.P.Thomas@lboro.ac.uk

Table 1 Summary of chemicals used in the experimental procedure.

Compound	IE/ eV	PA/ kJ.mol^{-1}	T_{Bp}/ °C	CAS	Formula
Methanol	10.84	754.3	64.7	67-56-1	CH_3OH
Ethanol	10.48	776	72.6	64-17-5	C_2H_5OH
Ethylene glycol	10.55	815	197.3	107-21-1	$C_2H_6O_2$
1,3-propandiol	10.80	876.2	182.2	57-55-6	$C_3H_8O_2$
(1)GHB	n.f.	n.f.	295.6	591-81-1	$\{C_4H_7O_3\}^-\{NH_4\}^+$

Note: IE: ionization energy, PA: proton affinity, T_{Bp}: boiling point. n.f: data not found.

(1) Obtained as a methanolic solution of concentration 1 mg.cm^{-3} in CH_3OH.

Table 2. Top: DMS factor levels selected for response optimisation study,

Bottom operational parameters selected (predicted from multiple linear regression)

Ref	E_d/kV.cm^{-1}	T/℃	N	δt/ms	Ref	E_d/kV.cm^{-1}	T/℃	N	δt/ms
1	20	80	50	10	14	25	120	50	50
2	20	80	50	50	15	25	120	100	10
3	20	80	100	10	16	25	120	100	50
4	20	80	100	50	17	22.5	100	75	30
5	20	120	50	10	18	22.5	100	75	30
6	20	120	50	50	19	20	100	75	30
7	20	120	100	10	20	25	100	75	30
8	20	120	100	50	21	22.5	80	75	30
9	25	80	50	10	22	22.5	120	75	30
10	25	80	50	50	23	22.5	100	50	30
11	25	80	100	10	24	22.5	100	100	30
12	25	80	100	50	25	22.5	100	75	10
13	25	120	50	10	26	22.5	100	75	50

Compound	E_d/kV.cm^{-1}	T/℃	N	δt/ms
Methanol	25	100 (108)	110 (60)	10 (48)
Ethanol	18	100 (80)	110 (60)	10 (50)
Ethylene glycol	23	100 (100)	110 (75)	10 (10)
1,3-propandiol	23	100 (120)	110 (75)	10 (30)
GHB	21	100	110	10

Note. E_d is the dispersion field (Some instruments use the term Radiofrequency Voltage.); T is the gas temperature within the ion filter, sometimes referred to as "cell" temperature; N is the number of steps in the differential mobility compensation field scan (defining the fidelity of the spectral features); and, δt is the dwell time for each step in the compensation field scan (defining the sensitivity of the response). The combination of N and δt defines the chromatographic performance of the system.

The heating and cooling rates of the DMS cell were too slow to enable multiple levels to be selected within a single chromatographic run. Further, switching the number of steps and step duration in the DMS spectra during a chromatographic run was not possible. Consequently mid-range levels were used.

Table 3 Instrument parameters.

Parameter	GC-DMS	TD-GC-DMS	Units
Gas chromatograph conditions			
Carrier gas	He	He	
Injection temperature	200		°C
Split flow	10.2	see below	$cm^3.min^{-1}$
Carrier gas flow	1.5	1.5	$cm^3.min^{-1}$
Carrier gas pressure	172	172	kPa
Phase	trifluoropropylmethylpolysiloxane		
Column length	30	30	m
Column diameter	0.32	0.32	mm
Phase thickness	0.5	0.5	µm
Temperature start	30	30	°C
Hold-time start	1.5	1.5	min
Temperature ramp-1	6	6	°C. min^{-1}
End temperature-1	60	60	°C
Hold-time-1	2	2	min
Temperature ramp-2	20	20	°C. min^{-1}
Temperature final	180	180	°C
Hold time final	2	10	min
Differential mobility spectrometry			
Transport gas	N_2	N_2	
Dispersion field frequency	1.2	1.2	MHz
Dispersion field mark space ratio	1:3	1:3	
[H2O] in transport gas	22.5 to 26.3	22.5 to 26.3	$mg.m^{-3}$
Transport gas flow rate	300	300	$cm^3.min^{-1}$
DMS cell temperature	100	100	°C
Compensation field scan range	-500 to 100	-500 to 100	$V.cm^{-1}$
Compensation field scan increment	109.1	109.1	$V.cm^{-1}$
Compensation field scan dwell-time	10	10	ms
Dispersion field start	25	25	$kV.cm^{-1}$
Dispersion field start hold time	0 to 125	0 to 125	s
Dispersion field step-1	18	18	$kV.cm^{-1}$
Dispersion field step-1 hold time	125 to 185	125 to 185	s
Dispersion field step-2	23	23	$kV.cm^{-1}$
Dispersion field step-2 hold time.	185 to 600	185 to 600	min
Thermal Desorption			
Tube purge duration		1	min
Tube purge flow		32	$cm^3.min^{-1}$
Tube purge temperature		35	°C
Primary desorption temperature		180	°C
Primary desorption split		0	$cm^3.min^{-1}$
Primary desorption time		5	min
Cold trap low temperature		0	°C
Secondary desorption temperature		300	°C
Secondary desorption split		12	$cm^3.min^{-1}$
Secondary desorption time		5	min

Table 4. Summary of the concentration ranges selected for the calibration of the DMS ($[i]_{(liq)}$) and the subsequent aliquot volumes ($V_{(S)}$) and the concentrations of the spiked saliva standards $[i]_{(s)}$ used to characterise the recovery of the analytes from by TD-GC-IMS

Compound	$[i]_{(liq)}$ / mg.dm^{-3}	B_0 / V.s	B_1/V.s.ng-1	LoD*/ng	R^2
Methanol	10 to 250	-0.02	0.27	0.42	0.994
Ethanol	250 to 1000	-8.22	0.28	4.62	0.983
Ethylene glycol	100 to 500	-0.02	0.27	0.52	0.994
1,3-propandiol	100 to 500	-0.48	0.30	1.42	0.997
GHB	20 to 500	-0.18	0.31	0.63	0.995

Note: these ranges relate to the linear portion of the calibration range where the integrated peak volume (I) was given by:

$$\boldsymbol{I(\mathrm{V.s}) = B_0(\mathrm{V.s}) + B_1\left(\mathrm{V.s.ng^{-1}}\right)};$$

*, estimated from linear regression.

Figure 1

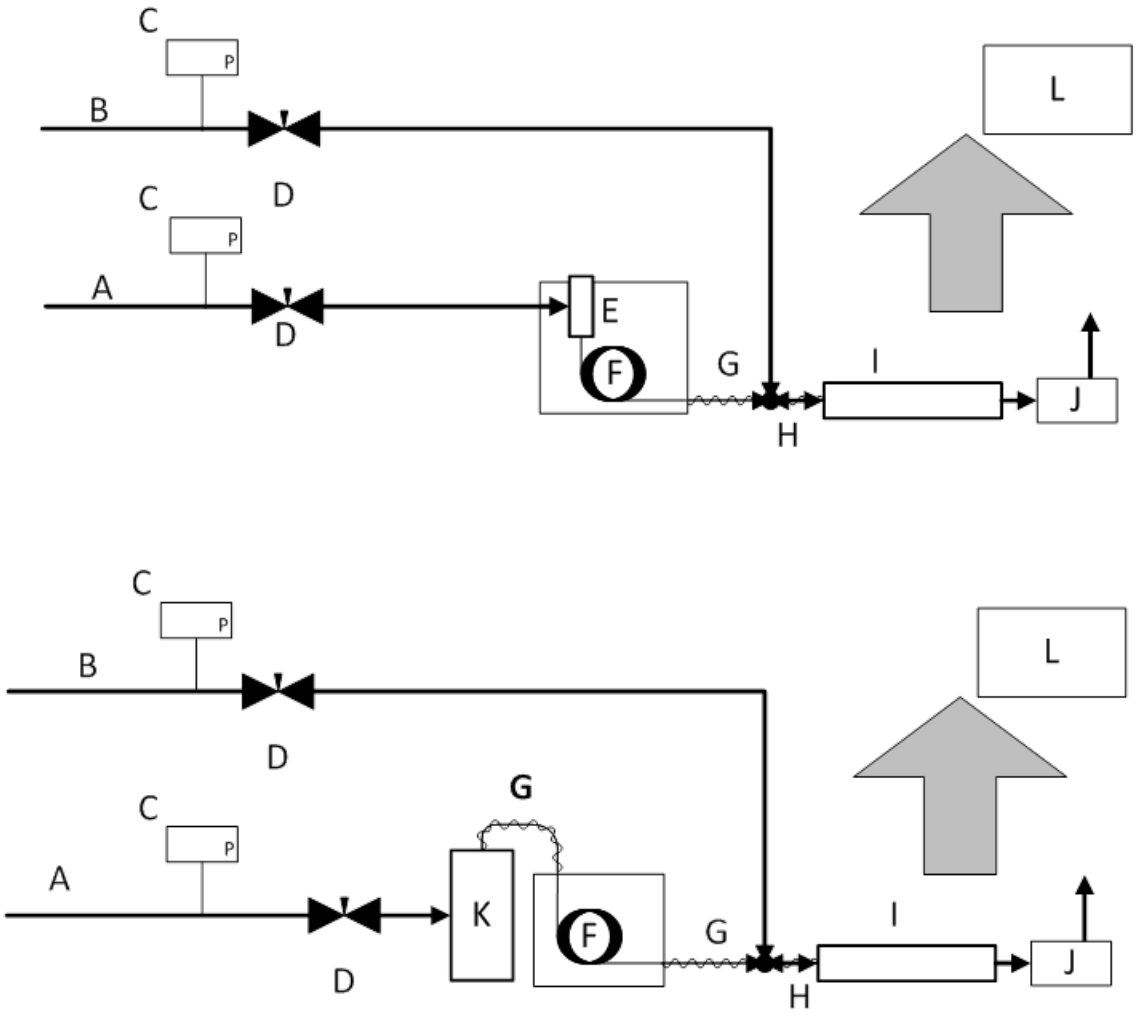


Figure 2

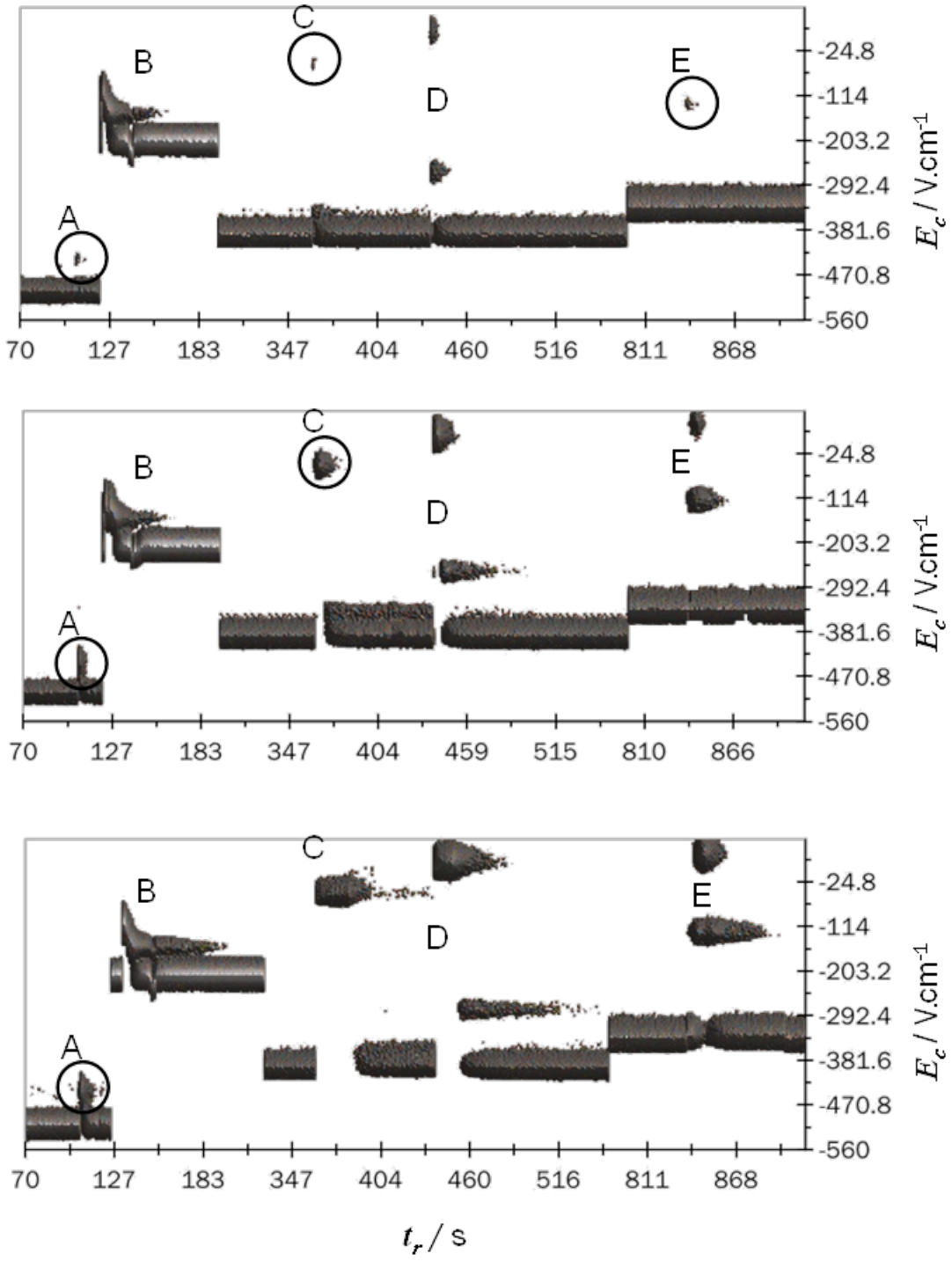

A
B
C
D
E
-24.8
-114
-203.2
-292.4
-381.6
-470.8
-560
E_c / V.cm^{-1}
70
127
183
347
404
460
516
811
868
459
515
810
866
t_r / s

Figure 3

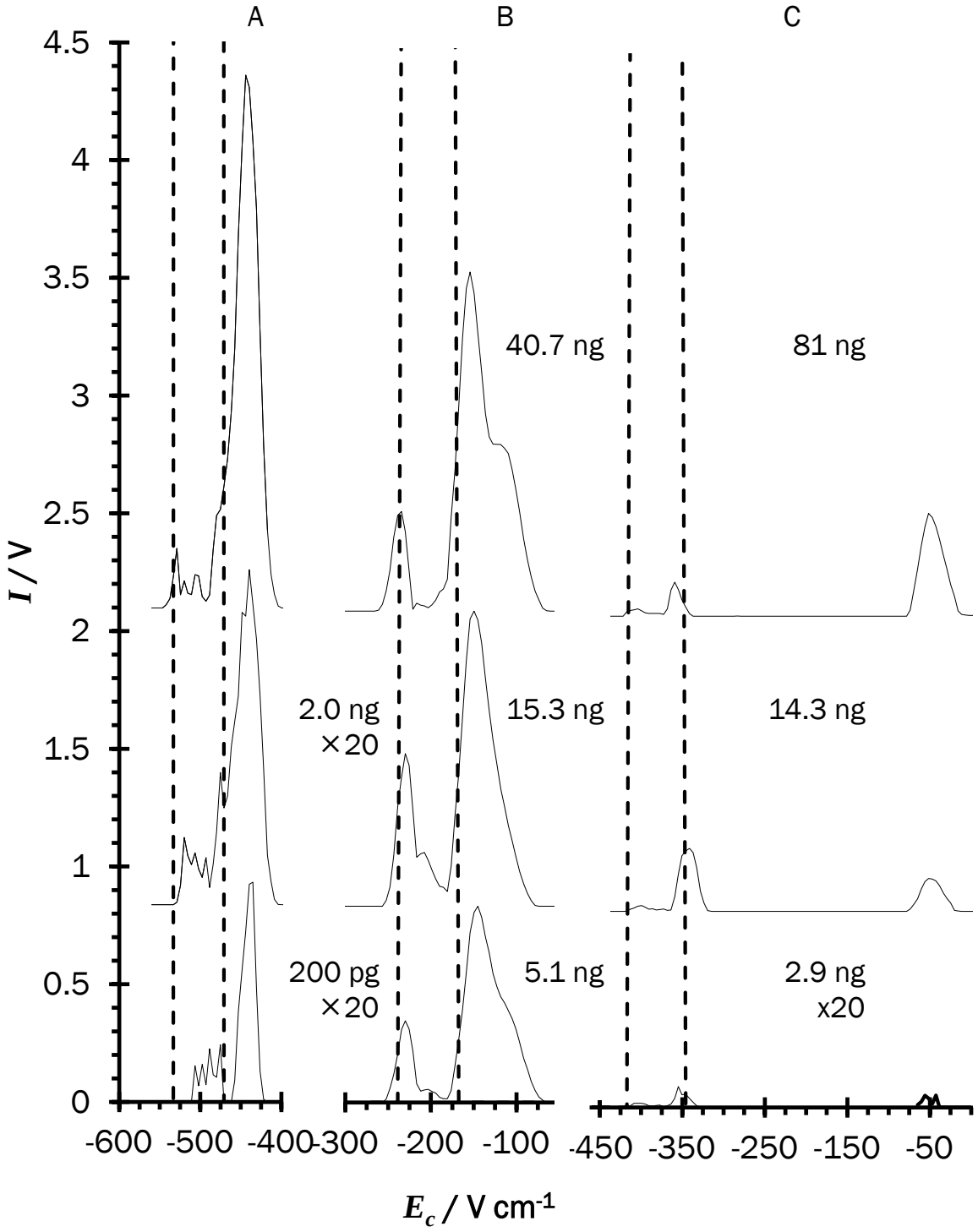

A
B
C
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
I / V
40.7 ng
81 ng
2.0 ng
×20
15.3 ng
14.3 ng
200 pg
×20
5.1 ng
2.9 ng
x20
-600 -500 -400
-300 -200 -100
-450 -350 -250 -150 -50
E_c / V cm-1

Figure 4

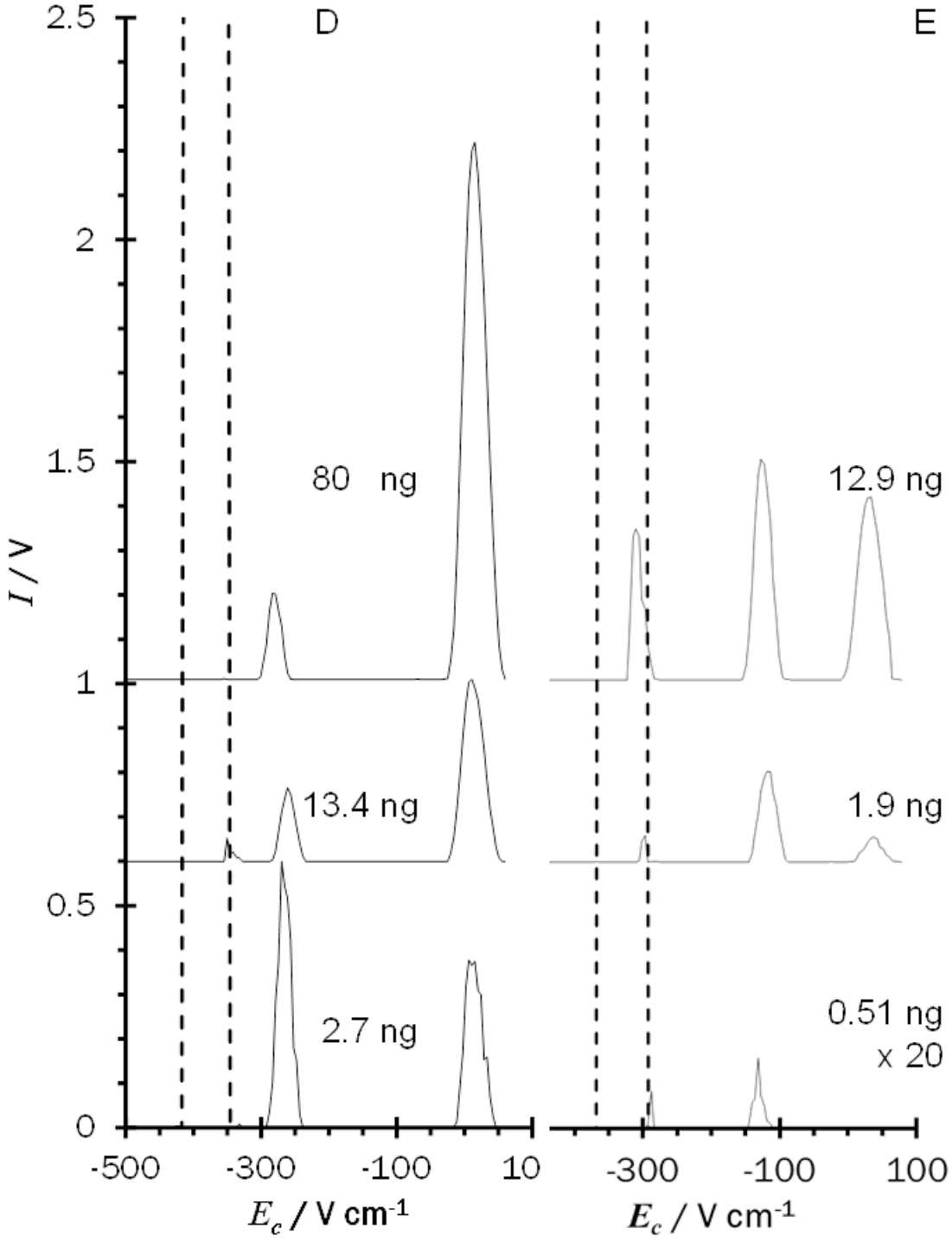

D
E
2.5
2
1.5
1
0.5
0
I / V
80 ng
13.4 ng
2.7 ng
12.9 ng
1.9 ng
0.51 ng
x 20
-500
-300
-100
10
-300
-100
100
E_c / V cm-1
E_c / V cm-1

Figure 5

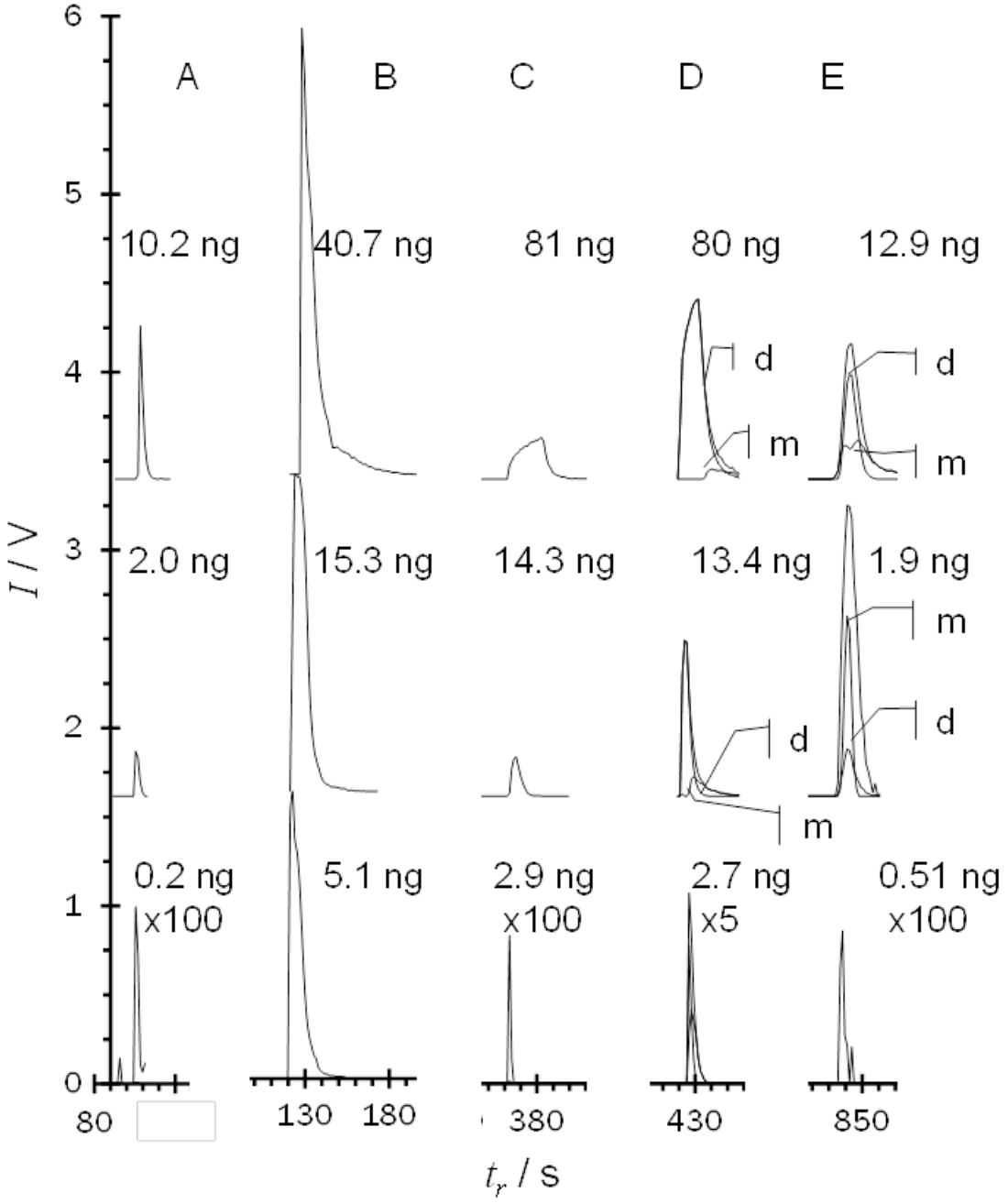

A
B
C
D
E
10.2 ng
40.7 ng
81 ng
80 ng
12.9 ng
2.0 ng
15.3 ng
14.3 ng
13.4 ng
1.9 ng
0.2 ng
x100
5.1 ng
2.9 ng
x100
2.7 ng
x5
0.51 ng
x100
d
m
I / V
t_r / s
80
130
180
380
430
850

Figure 6

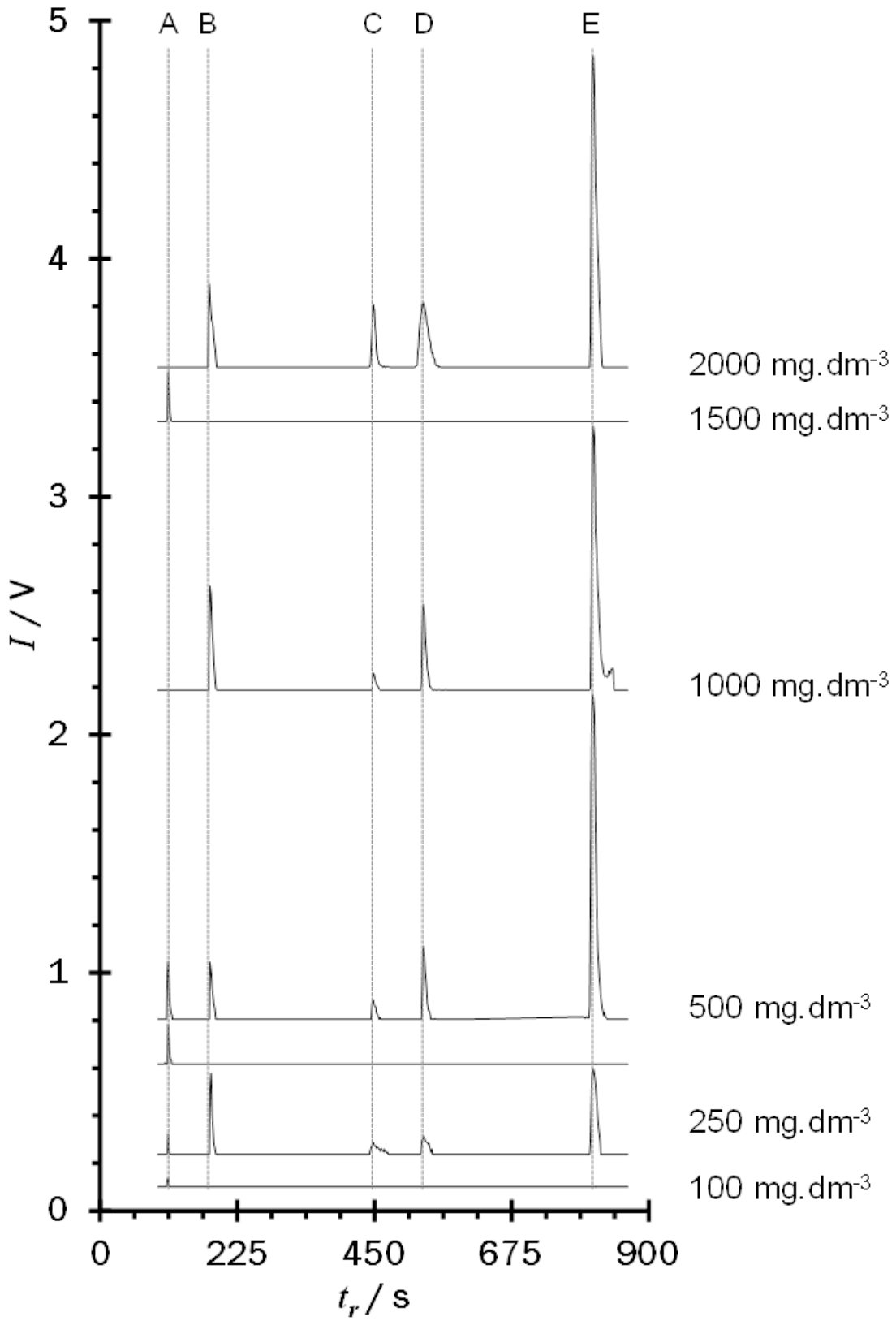
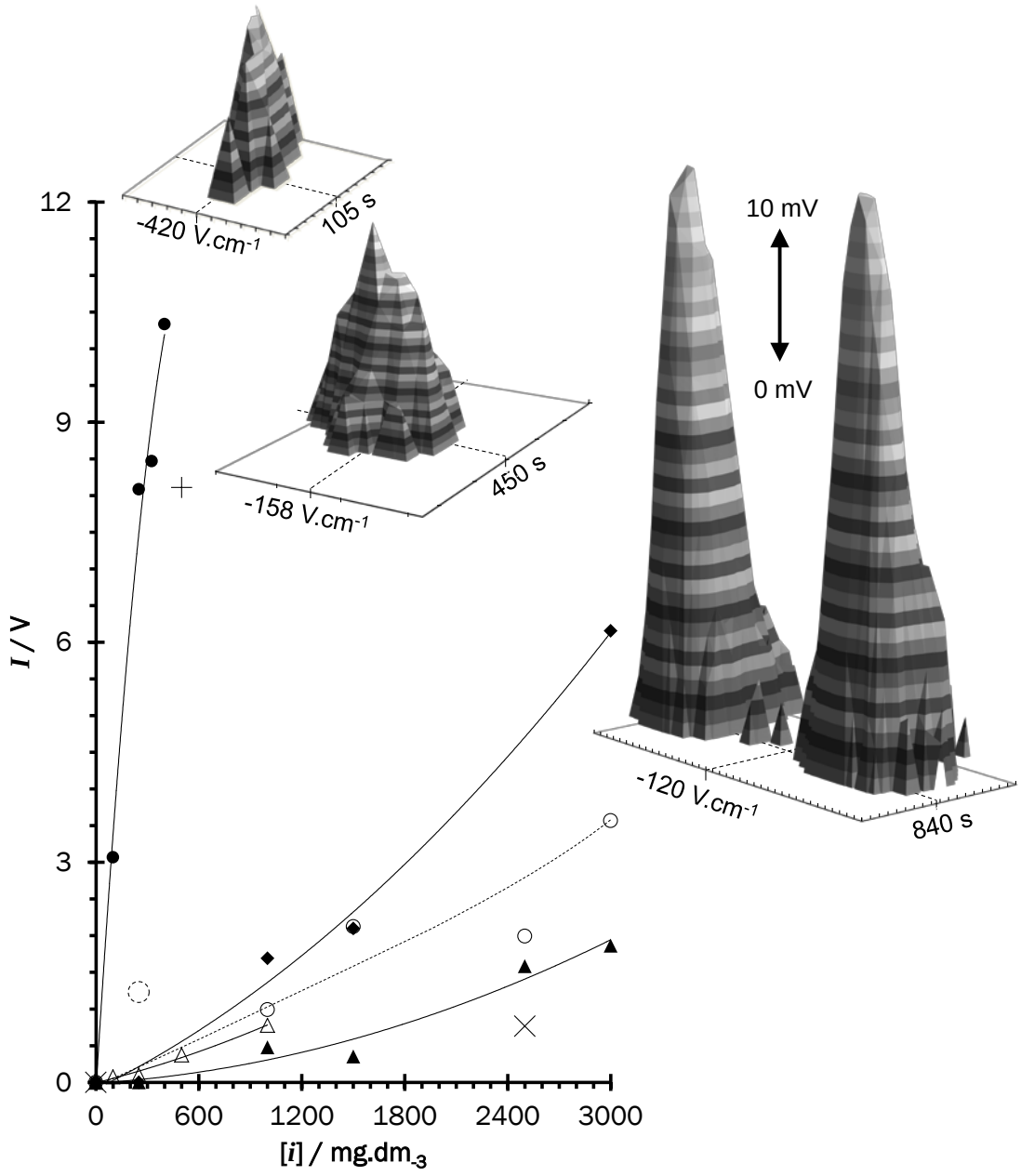

A
B
C
D
E
5
4
3
2
1
0
I / V
0
225
450
675
900
t_r / s
2000 mg.dm^{-3}
1500 mg.dm^{-3}
1000 mg.dm^{-3}
500 mg.dm^{-3}
250 mg.dm^{-3}
100 mg.dm^{-3}

Figure 7


12
9
6
3
0
I / V
0
600
1200
1800
2400
3000
[i] / mg.dm-3
-420 V.cm-1
105 s
-158 V.cm-1
450 s
10 mV
0 mV
-120 V.cm-1
840 s