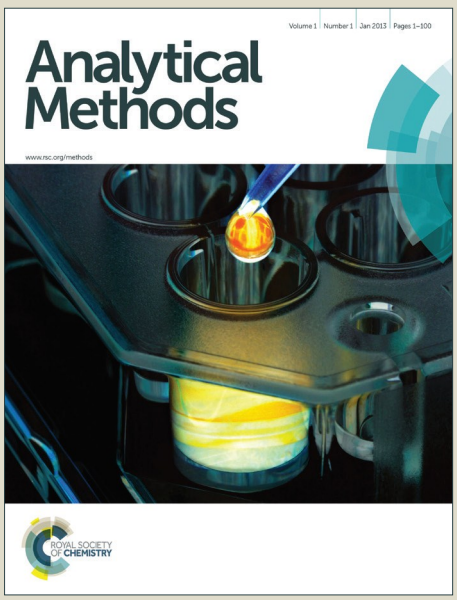
Analytical Methods

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Analytical Methods

RSCPublishing

PAPER

Thermal Desorption Ion Mobility Spectrometer for Measurement of Anticoagulant Rodenticide Diphacinone in Beverages via In-situ Acid-assisted Conversion

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2014,
Accepted 00th January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/

Liying Peng,[a,b] Xin Wang,[a] Wendong Chen,[a,b] Qinghua Zhou,[a,b] Weiguo Wang,[a] Haiyang Li *,[a]

Anticoagulant rodenticide diphacinone (DPN) with common form of sodium diphacinone (SDPN), playing an important role in controlling field rodent and mice in homes, is harmful to both human beings and domestic animals while taken mistakenly or poisoned intentionally. In this work, we proposed a simple and rapid method based on thermal desorption ion mobility spectrometry (TD-IMS) for detecting DPN and SDPN. SDPN, with ultra-low vapour pressure, was essentially measured in the form of DPN after its in-situ conversion with assistance of acid. Under the optimal conditions, the limits of detection (S/N = 3) for SDPN and DPN were less than 0.15 ng μL^{-1} while their recoveries were 96% and 97%, respectively, and the relative standard deviation for five measurements was less than 6.15%. Finally, the current method was used to detect the DPN and SDPN in beverages including green tea, cola, and coffee, demonstrating its capacity in the application for actual samples.

Introduction

Rodenticide diphacinone (DPN) and sodium diphacinone (SDPN, the common form of DPN), the first generation of indandione anticoagulant rodenticides, play an important role in controlling field rodent and mice in homes and have been reported to be hazardous to predatory and scavenging birds on a worldwide scale.[1-3] Empirical formulas of DPN and SDPN are $C_{23}H_{15}O_3H$ and $C_{23}H_{15}O_3Na$ with molar mass of 340.36 and 362.36 g mol^{-1}, respectively, and their structures were presented in Fig. 1. Due to the potential toxicity, it is dangerous if these two compounds are taken mistakenly or poisoned intentionally, particularly for children and other non-target species.[4-6] The acute median lethal dose for rats is 2 mg kg^{-1} and that for adult is 0.5-5 g, and even the intake of 0.06-0.25 g can cause poisoning.[7, 8] Moreover, these two compounds can be cumulated in the living body, and small amounts of feeding over several days to a week can cause death, blocking the identification of poison in time.[9, 10] Whereas, in many criminal cases, it is important for forensic scientists to discover the rodenticide DPN or SDPN in real samples in time, so that they can establish a chain of evidence and link criminals to the crime scenes of poisoning.[11, 12]

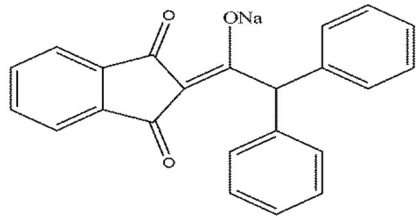


Fig. 1. Chemical schematic of DPN and SDPN.

Many traditional methods have been performed on the detection of trace anticoagulant rodenticides, including spectrophotometry,[13, 14] gas chromatography-mass spectrometry (GC–MS),[15] liquid chromatography with ultraviolet detection (LC)[16] and liquid chromatography-mass spectrometry (LC–MS).[17, 18] However, the limit of detection (LOD) of spectrophotometric method for anticoagulant rodenticides is

about 20 μM, which is not suitable for the trace detection. For those methods involving chromatography techniques above, a complicated pretreatment procedure is usually required and the analysis time is relatively long, hampering their application in fast detection. What's more, it is notable that the traditional methods are almost aimed for DPN detection but not for its salt form (SDPN). Thus, a rapid analytical method is desired to certify the suspected poisoning of SDPN as well as DPN for both domestic animals and human beings in accidental and intentional cases.[19]

Ion mobility spectrometry (IMS) is a separated technique for gaseous ions basing on their different mobilities in an weak electric field.[20] With the advantages of simple even no sample pretreatment, high sensitivity, rapid analysis speed, and good portability,[21] IMS is commonly employed for the detection of toxic industrial compounds,[22-24] chemical warfare agents,[25] explosives,[26, 27] narcotic drugs [28-30] and illicit addition in food.[31]

In order to accomplish the IMS detection of liquid and solid samples, some vaporization pretreatments are usually used,[32] among which the thermal desorption is one of the simplest way.[33] Currently, the thermal desorption ion mobility spectrometry (TD-IMS) is being deployed for rapid on-site screening, such as explosives and narcotics.[34, 35] In this technique, analytes on a sampling swab are thermally desorbed by rapid heating (typically 230 °C to 280 °C), producing corresponding vapor molecules for further IMS analysis. Nevertheless, it still remains a great challenge for TD-IMS to detect non-volatile salt such as SDPN owing to their ultra-low vapor pressure. Therefore, in this work, TD-IMS with in-situ acid-assisted conversion was developed for the fast detection of rodenticide DPN and SDPN. After the optimization of acid kinds, desorber temperature and tube temperature, this method was implemented for analysis of SDPN and DPN in different beverages.

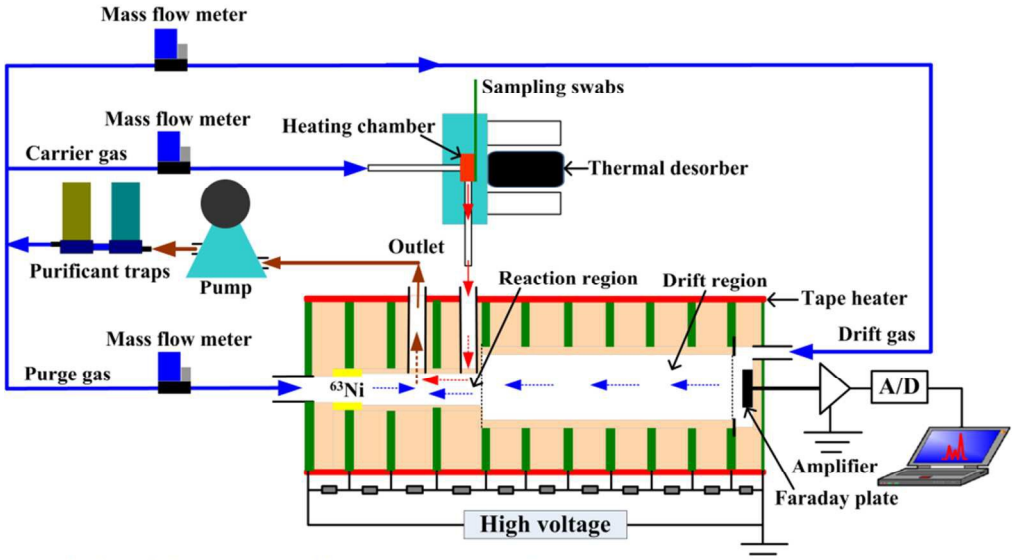


Fig. 2. Schematic diagram of the thermal desorption ion mobility spectrometry (TD-IMS).

Materials and methods

Apparatus

The TD-IMS used in this study was constructed in our laboratory, which consisted of a thermal desorber, a radioactive ^{63}Ni ionization source, a reaction region, a Bradbury-Nielsen (BN) gate, a drift region, an aperture grid, a Faraday plate, and an amplifier, as shown in Fig. 2.[36] In the reaction region, the inlet at the vicinity of BN gate was connected with a thermal desorber for sample introduction, and the ^{63}Ni source was flushed by a purge gas to protect it from acid corrosion. In the test, the analytes vaporized from the thermal desorber were sent into the ionization region by a carrier gas. Air, dried by purificant traps containing silica gel, activated carbon and 13X molecular sieve, was divided into drift, carrier and purge gases via mass flow meters (Beijing Sevenstar Electronics Co., Ltd.). The IMS apparatus was operated in the negative mode, and the operating parameters were listed in Table 1.

Table1. Operational conditions for TD-IMS.

Operating condition	Setting
Ionization source	^{63}Ni
Drift region length	6.5 cm
Drift field	225 V cm^{-1}
Shutter grid pulse width	300 μs
Tube temperature	110 °C
Desorber temperature	210 °C
Flow of drift gas	800 mL min^{-1}
Flow of carrier gas	400 mL min^{-1}
Flow of purge gas	400 mL min^{-1}

Chemicals and reagents

DPN and SDPN (analytical grade) were offered by Dalian Test Chemical Plant (Dalian, China). Methanol, phosphoric acid (H_3PO_4), hydrochloric acid (HCl) and acetic acid (CH_3COOH) used in experiments was analytical grade and purchased from Tianjin Chemical Reagent Factory (Tianjin, China). Beverages including green tea, cola and coffee were purchased from a

local supermarket and stored in a refrigerator (4 °C) until required. Distilled water was used throughout the experiment.

Methods and calculations

500 ng μL^{-1} DPN and SDPN standard stock solutions were prepared by weighing sample and dissolving them in methanol. 500 ng μL^{-1} DPN and SDPN test stock samples of green tea, cola and coffee were prepared by directly adding a known amount of DPN and SDPN into corresponding beverage. The standard and test samples were obtained by gradually diluting the relevant stock solutions with distilled water and corresponding beverage.

1 mL SDPN samples were acidized by adding 15 μL 3 % acid including H_3PO_4, HCl, CH_3COOH, followed by an ultrasonic treatment for 3 min. Then, 5 μL sample solution were deposited on the sampling swabs (Teflon coated fiber glass, 2 cm width × 8 cm length), after the solvent was evaporated, the swabs were inserted into the thermal desorber for IMS analysis.

Reduced mobility, K_0 (cm^2 s^{-1} V^{-1}) of analytes can be calculated by Eq. (1) , where K_{0a} is the reduced mobility of the analyte, t_{ds} and t_{da} is the drift time of the standard and the analyte, respectively.[37, 38] In this work, TNT with K_0 of 1.54 cm^2 s^{-1} V^{-1} at 110 °C was used as the standard.

$$K_{0a} = K_{0s} \times (t_{ds}/t_{da}) \quad (1)$$

Results and discussion

Detection of SDPN via in situ acid-assisted conversion

From the ion mobility spectra of the background and 5 μL 10 ng μL^{-1} DPN standard solution in Fig. 3, we can observe the prominent ion peak for DPN with K_0 of 1.04 cm^2 s^{-1} V^{-1}. However, when the same amount of SDPN standard solution was directly detected by TD-IMS, no product ion peaks can be found, which might be attributed to its ultra-low vapor pressure.

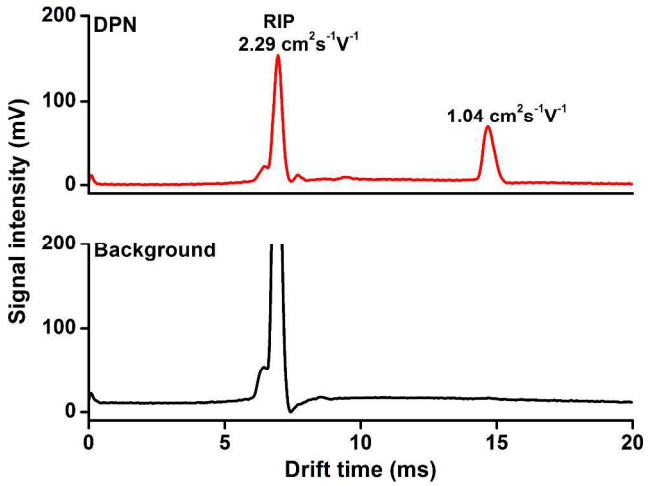


Fig. 3. Ion mobility spectra recorded for background and 5 μL 10 ng μL^{-1} DPN.

DPN is the corresponding acid of SDPN, thus, it might be a potential method to detect SDPN by TD-IMS after its conversion to DPN with assistance of acid. In order to confirm this hypothesis, three common acids including H_3PO_4, HCl and CH_3COOH were chosen to in-situ acidize the SDPN samples, and their corresponding ion mobility spectra were shown in Fig. 4(a). From the spectra, it is clear that no ion peaks can be observed for SDPN acidified with CH_3COOH, whereas, when the SDPN solution was acidified with H_3PO_4 or HCl, an ion peak (K_0 = 1.04 cm^2 s^{-1} V^{-1}) can be readily detected, which was the same as that of DPN. And more, the matrix interference from H_3PO_4 was much less than that of HCl, and the peak intensity (K_0 = 1.04 cm^2 s^{-1} V^{-1}) using H_3PO_4 was about 1.7 times higher than that obtained using HCl, therefore H_3PO_4 solution was chosen for the acid conversion in the following experiments.

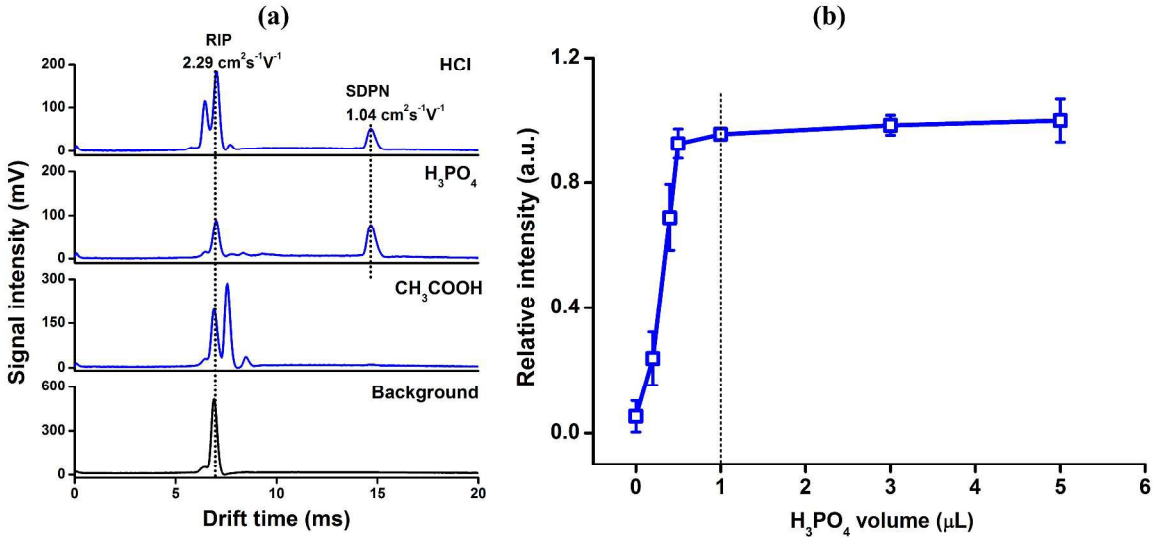


Fig. 4. (a) Detection of 5 μL 10 ng μL^{-1} SDPN with TD-IMS by using H_3PO_4, HCl and CH_3COOH to perform acidification, respectively; (b) the variations of SDPN response versus H_3PO_4 volume added in 1 mL sample solution

To optimize the acidification degree, we prepared 1 mL 10 ng μL^{-1} SDPN standard solutions by adding different volumes of 3% H_3PO_4 solution, and the peak intensities for 5 μL samples were illustrated in Fig. 4(b), from which we can see

that the dosage of 1 μL H_3PO_4 solution promised the best response of SDPN, afterward, its response remained almost the same. Whereas, taking account of PH differences in beverages, 15 μL 3% H_3PO_4 was added into 1 mL real samples to ensure the efficient conversion of SDPN to DPN.

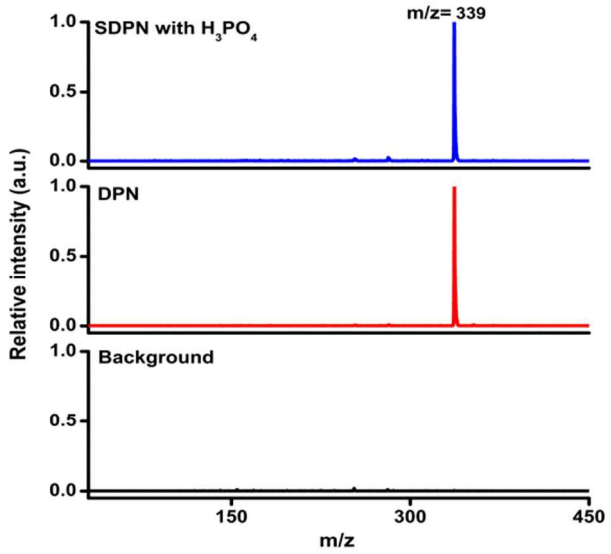


Fig. 5. Ion patterns of DPN and SDPN with H_3PO_4 acidification in the negative ^{63}Ni source identified with a homemade ion trap mass spectrometer.

Demonstrating the conversion of SDPN to DPN by ion trap mass spectrometer (ITMS)

In order to demonstrate the conversion of SDPN to DPN via acidification, the product ions of DPN and SDPN in negative ^{63}Ni source were studied by a homemade ion trap mass spectrometer (ITMS). As depicted in Fig. 5, the product ion for DPN was assigned to $C_{23}H_{15}O_3^-$ at m/z = 339; but no product ions can be detected for SDPN without any treatment, which was quite similar to result of IMS experiment. When the SDPN solution was acidified with H_3PO_4, a product ion peak at m/z = 339 was observed in the mass spectrum, well proving the reaction of SDPN with hydrogen ion (H^+) in solution to produce DPN. Therefore, both DPN and SDPN could be measured by quantifying of $C_{23}H_{15}O_3^-$.

Optimizing the temperature of desorber and drift tube

Fig. 6(a) presented the effect of thermal desorber temperature on the detection of DPN and SDPN, from which we can see that as the temperature was increased from 170 to 210 °C, more samples could be evaporated into the gas phase, resulting in an enhancement of responses for 5 μL 10 ng μL^{-1} DPN and SDPN, Nevertheless, a higher temperature would release more interfering substances, which would competitively react with reactant ions and decrease the response of DPN and SDPN. Hence, the thermal desorber temperature of 210 °C was selected as the optimum temperature.

Plotting the peak intensities of 5 μL 10 ng μL^{-1} DPN and SDPN versus drift tube temperature in Fig. 6(b), it is clear that the peak intensities of DPN and SDPN gradually increased to the maximum at 110 °C and then slightly decreased as the temperature was further increased. With the increase of drift tube temperature, the shorter drift time of DPN and SDPN was trended to increase their responses due to the decreasing ion-molecule collisions in drift region, while the enhanced radial diffusion in drift region would boost the ion loss, so it was understandable that an optimized drift tube temperature of 110 °C can be observed.

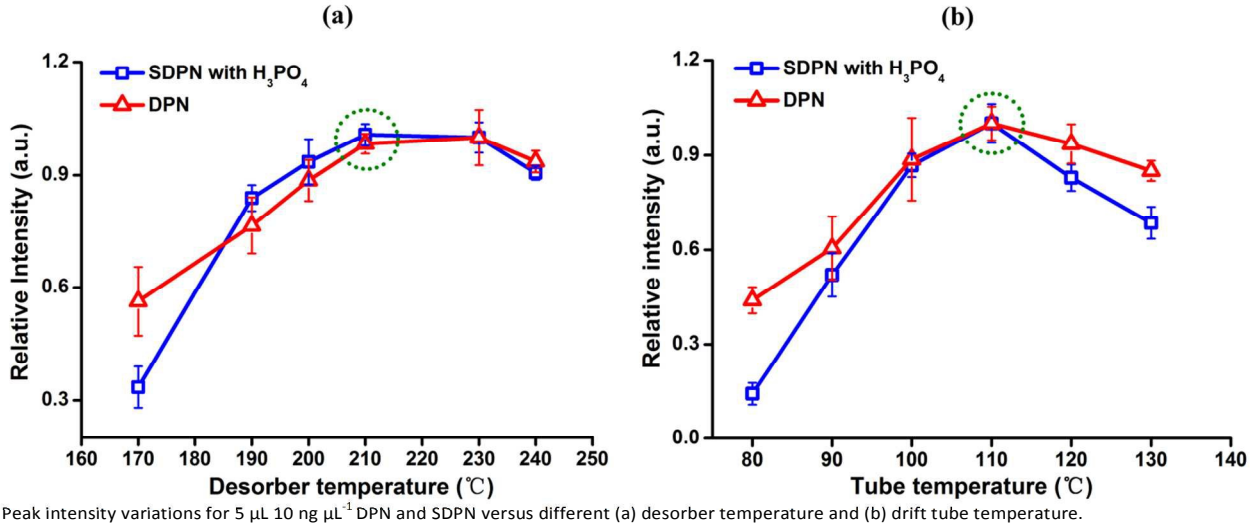


Fig. 6. Peak intensity variations for 5 μL 10 ng μL^{-1} DPN and SDPN versus different (a) desorber temperature and (b) drift tube temperature.

Quantitative analysis

Under the optimized conditions, the calibration curves of SDPN and DPN were obtained, and the quantitative results were listed in Table 2. The linear response range of SDPN and DPN were 2-20 ng μL^{-1} and 1-20 ng μL^{-1}, with LODs (S/N=3) of 0.15 and 0.13 ng μL^{-1}, respectively, and their relative standard deviation percentage (RSD) for 5 times measurement of 5 μL 10 ng μL^{-1} SDPN and DPN was 6.15% and 3.47%, respectively. The recovery rate of the detection of 10 ng μL^{-1} SDPN and DPN

added in water was 96% and 97%, respectively, indicating a satisfactory repeatability for measurement.

Table 2 Quantitative results for DPN and SDPN.

	Calibration curves	R^2	linear range (ng μL^{-1})	LOD (ng μL^{-1})	RSD (%)
DPN	y=4.98x+20.61	0.977	1-20	0.13	3.47
SDPN	y=4.15x+24.32	0.961	2-20	0.15	6.15

Detection of DPN and SDPN in beverages

The ion mobility spectra of 5 μL beverage samples (cola, coffee and green tea) containing 20 ng μL^{-1} SDPN or DPN were shown in Fig. 7. Via in-situ acid-assisted conversion, SDPN in different beverage can be detected by its ion peak with K_0 of 1.04 cm^2 s^{-1} V^{-1}, as shown in Fig. 7(a), while DPN can be directly detected in Fig. 7(b). The peak intensity of SDPN in cola, coffee and green tea was 25, 26 and 30 mV, respectively, while that of DPN was 43, 45 and 76 mV. The ion peaks of other annexing agents such as sugar and honey in beverages were far away from the PIP, not disturbing the identification of DPN or SDPN. Their concentrations in actual samples could be qualified by standard addition. And the total amount of DPN and SDPN could be also measured by analysis the results with and without acid treatment.

Conclusions

In this work, via the pre-conversion of SDPN to DPN with acid-assistance, we accomplished the fast detection of trace rodenticide DPN and SDPN in complex matrixes such as common beverages by TD-IMS, and their LODs can be achieved sub ng μL^{-1} level. In future studies, we will apply the current acid-assisted TD-IMS method to measure DPN or SDPN in other matrixes, such as food and saliva, which might have stronger clinical and forensic relevance.

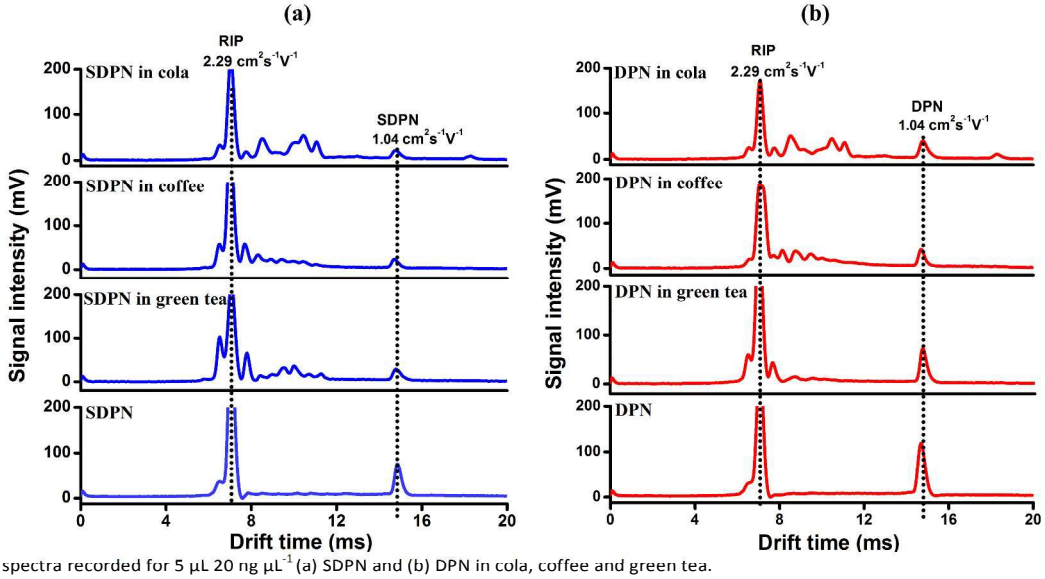


Fig. 7. Ion mobility spectra recorded for 5 μL 20 ng μL^{-1} (a) SDPN and (b) DPN in cola, coffee and green tea.

Acknowledgements

This work is partially supported by NSF of China (21077101, 20907052, 21177124, 21275143 and 21207129). We thank Miss Dandan Jiang and Dr. Chuang Chen for the contribution to the data processing; we thank Prof. Jinghua Li for offering the drugs and reagents.

Notes and references

[a] Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, 116023, People's Republic of China. E-mail: hli@dicp.ac.cn; Fax: +86 411 84379517.

[b] Graduate University of Chinese Academy of Sciences, Beijing, 100039, People's Republic of China.

1. J. Tang, S. Qi and X. Chen, *J. Mol. Struct.*, 2005, **779**, 87-95.
2. B. A. Rattner, K. E. Horak, S. E. Warner, D. D. Day, C. U. Meteyer, S. F. Volker, J. D. Eisemann and J. J. Johnston, *Environ. Toxicol. Chem.*, 2011, **30**, 1213-1222.
3. B. A. Rattner, K. E. Horak, R. S. Lazarus, K. M. Eisenreich, C. U. Meteyer, S. F. Volker, C. M. Campton, J. D. Eisemann and J. J. Johnston, *Ecotoxicology*, 2012, **21**, 832-846.
4. G. S. Bumbrah, K. Krishan, T. Kanchan, M. Sharma and G. S. Sodhi, *Forensic Sci. Int.*, 2012, **214**, 1-6.
5. Q. Liu, L. Zhou, N. Zheng, L. Zhuo, Y. Liu and L. Liu, *Forensic Sci. Int.*, 2009, **193**, 88-94.
6. D. B. Sabine, *Microchem. J.*, 1964, **8**, 442-443.
7. J. J. Johnston, W. C. Pitt, R. T. Sugihara, J. D. Eisemann, T. M. Primus, M. J. Holmes, J. Crocker and A. Hart, *Environ. Toxicol. Chem.*, 2005, **24**, 1557-1567.
8. W. C. Pitt, M. Higashi and T. M. Primus, *Food Chem. Toxicol.*, 2011, **49**, 2030-2034.
9. R. T. Sterner, *Bull. Environ. Contam. Toxicol.*, 1979, **23**, 211-217.
10. M. Z. Mesmer and R. A. Flurer, *J. Chromatogr.*, 2000, **891**, 249-255.

11. A. Banasr, A. Benzarti, A. Mezni, M. Ayari and M. Hamdoun, *Forensic Sci. Int.*, 2003, **136**, 321-321.
12. X. H. Chen, M. Q. Cai and M. C. Jin, *Chromatographia*, 2009, **70**, 1201-1206.
13. H. Drechsler and P. Launer, *Monatsh. Veterinarmed.*, 1983, **38**, 225-228.
14. M. Cai, X. Chen and M. Jin, *J. Fluoresc.*, 2011, **21**, 1349-1356.
15. R. W. Bullard, G. Holguin and J. E. Peterson, *J. Agric. Food Chem.*, 1975, **23**, 72-74.
16. M. Jin, X. OuYang and X. Chen, *J. Appl. Toxicol.*, 2007, **27**, 18-24.
17. M. Jin, M. Cai and X. Chen, *J. Anal. Toxicol.*, 2009, **33**, 294-300.
18. L. J. Marek and W. C. Koskinen, *J. Agric. Food Chem.*, 2007, **55**, 571-576.
19. F. Papin, F. Clarot, C. Vicomte, J. M. Gaulier, C. Daubin, F. Chapon, E. Vaz and B. Proust, *Forensic Sci. Int.*, 2007, **166**, 85-90.
20. G. A. Eiceman, Z. Karpas, *CRC Press*, 1994.
21. S. Armenta, M. Alcala and M. Blanco, *Anal. Chim. Acta*, 2011, **703**, 114-123.
22. I. Marquez-Sillero, E. Aguilera-Herrador, S. Cardenas and M. Valcarcel, *Trac-trends Anal. Chem.*, 2011, **30**, 677-690.
23. C. Chen, C. Dong, Y. Du, S. Cheng, F. Han, L. Li, W. Wang, K. Hou and H. Li, *Anal. Chem.*, 2010, **82**, 4151-4157.
24. M. Nousiainen, S. Holopainen, J. Puton and M. Sillanpää, *Talanta*, 2011, **84**, 738-744.
25. R. P. Erickson, A. Tripathi, W. M. Maswadeh, A. P. Snyder and P. A. Smith, *Anal. Chim. Acta*, 2006, **556**, 455-461.
26. J. Arndt, S. Bell, L. Crookshanks, M. Lovejoy, C. Oleska, T. Tulley and D. Wolfe, *Forensic Sci. Int.*, 2012, **222**, 137-145.
27. J. S. Caygill, F. Davis and S. P. J. Higson, *Talanta*, 2012, **88**, 14-29.
28. Q. Zhou, W. Wang, H. Cang, Y. Du, F. Han, C. Chen, S. Cheng, J. Li and H. Li, *Talanta*, 2012, **98**, 241-246.
29. J. R. Verkouteren and J. L. Staymates, *Forensic Sci. Int.*, 2011, **206**, 190-196.
30. Q. Zhou, E. Li, X. Wang, Y. Gong, L. Hua, W. Wang, T. Qu, J. Li, Y. Liu, C. Wang and H. Li, *Anal. Methods*, 2014, **6**, 698.
31. M. Khalesi, M. Sheikh-Zeinoddin and M. Tabrizchi, *Talanta*, 2011, **83**, 988-993.
32. G. A. Eiceman, D. Young and G. B. Smith, *Microchem. J.*, 2005, **81**, 108-116.
33. X. Y. Liu, S. Nacson, A. Grigoriev, P. Lynds and J. Pawliszyn, *Anal. Chim. Acta*, 2006, **559**, 159-165.
34. M. Najarro, M. E. Dávila Morris, M. E. Staymates, R. Fletcher and G. Gillen, *Analyst*, 2012, **137**, 2614-2622.
35. C. Fuche and J. Deseille, *Actualite Chimique*, 2010, **342-3**, 91-95.
36. X. Liang, Q. Zhou, W. Wang, X. Wang, W. Chen, C. Chen, Y. Li, K. Hou, J. Li and H. Li, *Anal. Chem.*, 2013, **85**, 4849-4852.
37. C. L. Crawford, B. C. Hauck, J. A. Tufariello, C. S. Harden, V. McHugh, W. F. Siems and H. H. Hill, Jr., *Talanta*, 2012, **101**, 161-170.
38. S. Cheng, J. Dou, W. Wang, C. Chen, L. Hua, Q. Zhou, K. Hou, J. Li and H. Li, *Anal. Chem.*, 2013, **85**, 319-326.

Graphical Abstract

TD-IMS was constructed to fast detecting rodenticide diphacinone via in-situ acid-assisted conversion and applied for its on-site determination in beverages.