

# Paper spray ionization using non-polar solvents

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## Supporting Information

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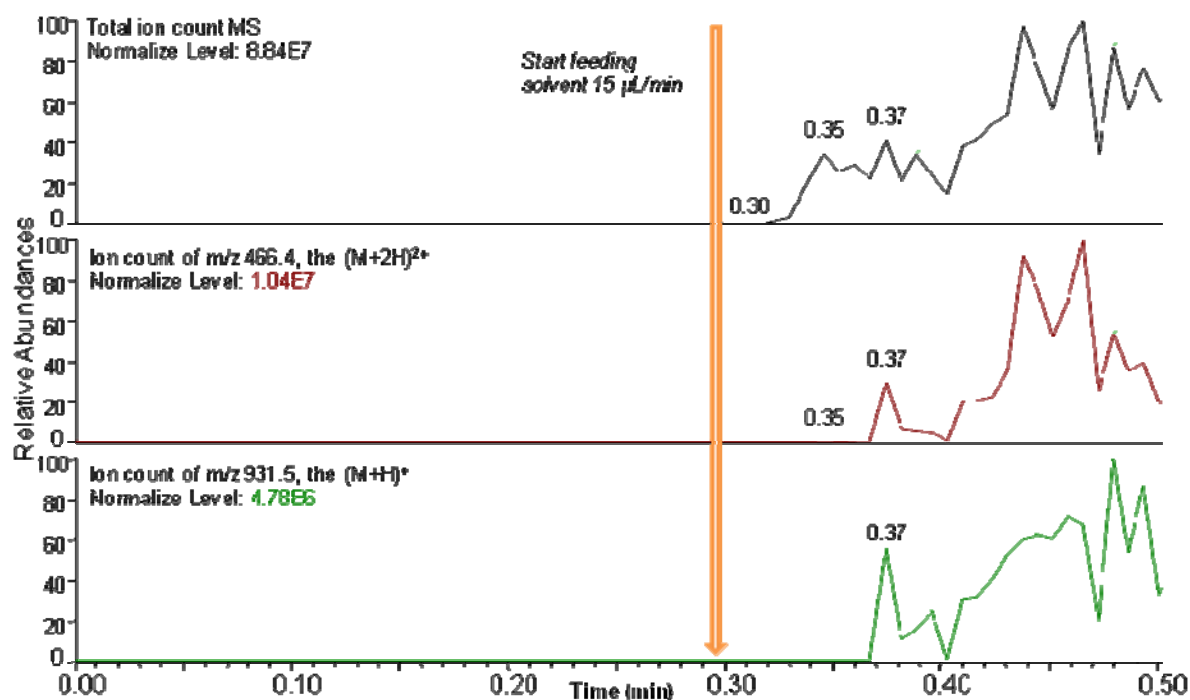
## 1. Experimental conditions

A Finnigan LTQ mass spectrometer (Thermo Electron, San Jose, CA) was used under the following instrumental conditions unless specified otherwise: capillary temperature: 150 °C; heated-capillary voltage: 15 V; tube-lens voltage: 65V. Paper spray voltage: 1~2 kV for both positive and negative modes; nanoESI voltage: 1.5 kV. The experimental set-up for paper spray was similar to that described in a previous publication<sup>1</sup> except that a 500  $\mu$ L syringe (Hamilton) driven by the syringe pump of the Finnigan LTQ was used to feed solvent, through a silica capillary (I.D. 100  $\mu$ m, O.D. 190  $\mu$ m) to the center of the paper triangle.

Solvents and chemicals were purchased from Sigma Aldrich (St. Louis, MO), and were used without further purification. The alkane solvents (hexane etc.) are all the normal isomers. The paper substrate used was Grade 1 chromatography paper purchased from Whatman (Maidstone, England). The PTFE membrane is Mitex Membrane Filter (Catalog #: LSWP) purchased from Millipore (Bedford, MA).

## 2. Typical experiment and ion chromatograms

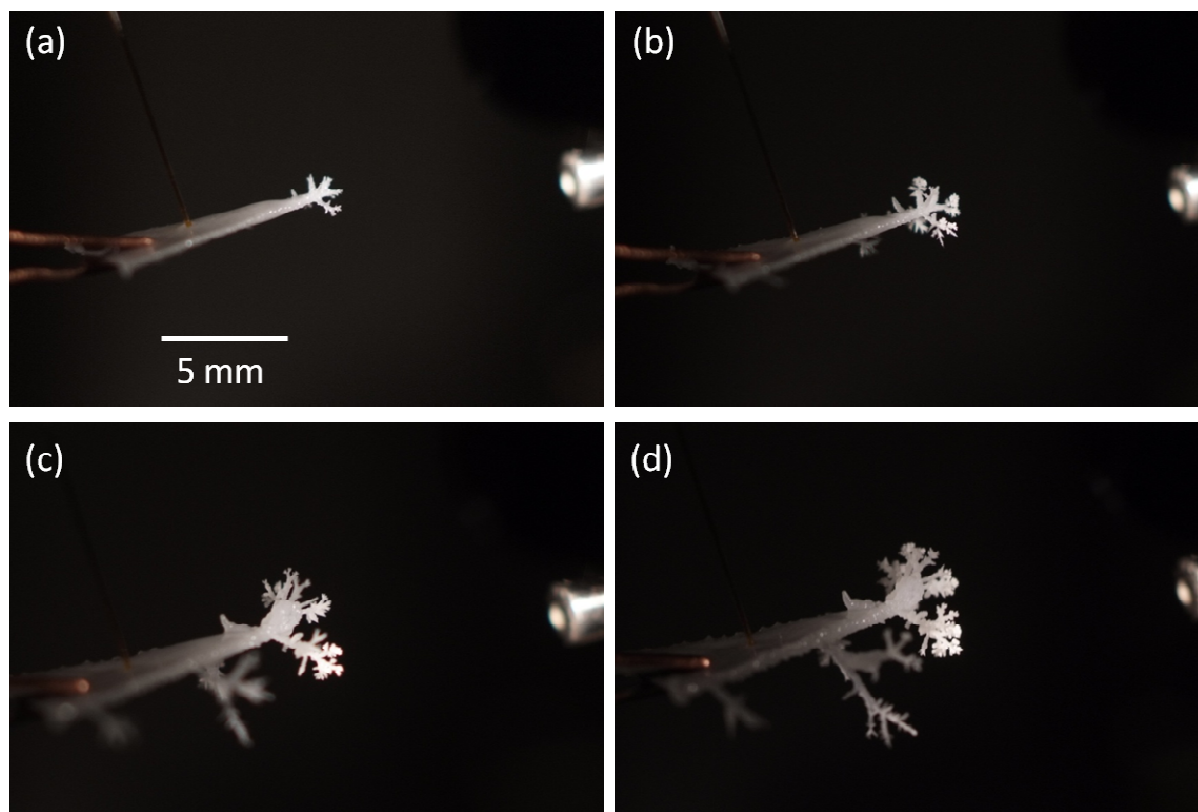
Samples were loaded onto paper in 1  $\mu$ L solution and allowed to dry for 5 minutes under ambient conditions. Applying voltage to the dried paper triangle did not generate ion signal (as shown by the total ion count chronogram, from 0 to 0.3 min in Fig. S1). Only when the solvent (hexane in this case) was also fed to the paper triangle (starting from 0.3 min), were analyte ions generated by paper spray and detected by the mass spectrometer.



**Figure S1.** Ion chromatogram showing total ion count (upper picture) and selected angiotensin III ions ( $m/z$  466,  $(M+2H)^{2+}$  and  $m/z$  931.5, the  $(M+H)^+$ ), in a typical paper spray experiment using n-hexane as the spraying solvent and  $1\mu\text{g}$  sample deposited on paper.

### **3. Ice formation using non-polar solvents in paper spray ionization**

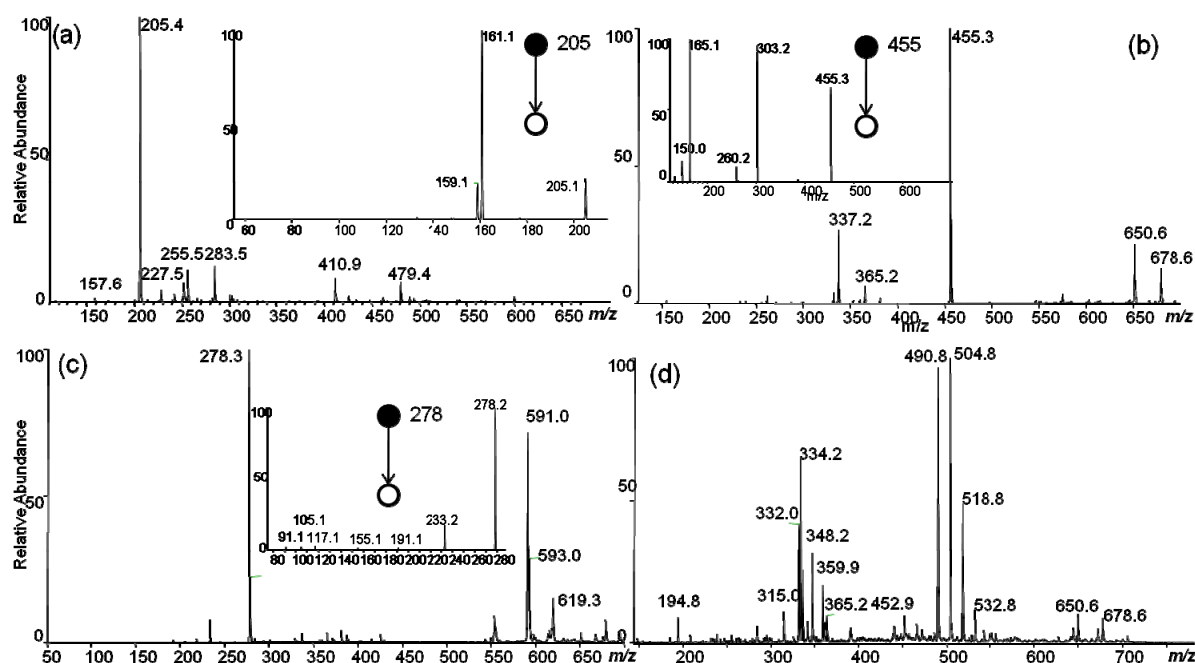
When n-pentane was used as the paper spray solvent, the droplets were too small to be visualized by light scattering. However, the fast evaporation of pentane condensed water vapor from the ambient atmosphere and left ice dendrites attached to the paper and apparently generated along the path of droplets (photos below). In comparison, when no voltage was applied, the evaporation of pentane cooled the paper triangle but it did not yield ice dendrites. It is assumed that similar effects occurred with hexane and other solvents. The condensation of water vapor on the paper surface might play an important role in the ionization process. It is possible that the condensed water provides an aqueous microenvironment near the tip where the analytes are extracted and field ionized. Preliminary experiments also show that it is difficult to get stable signals when the paper spray is attempted in a dry-air environment.



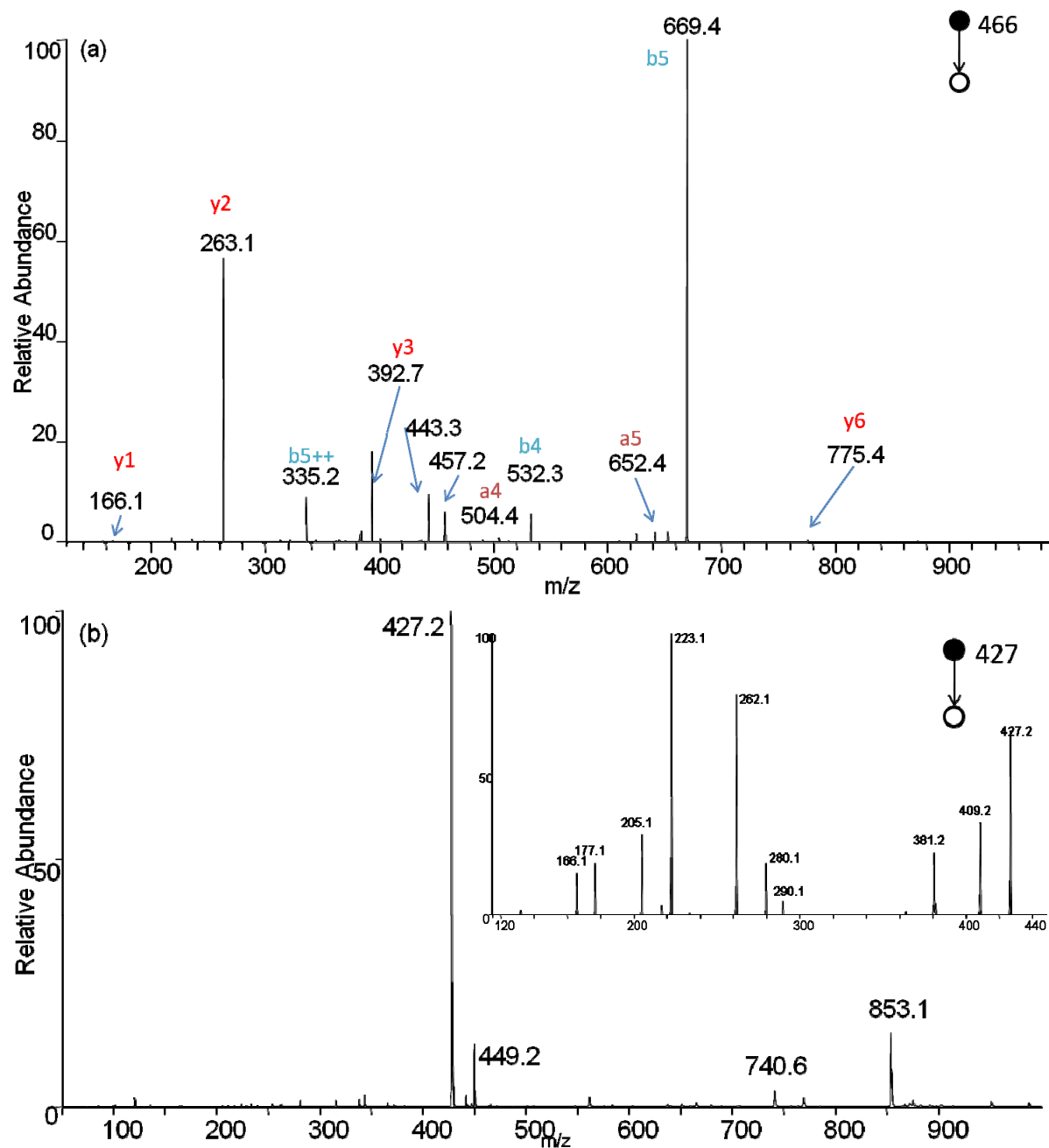
**Figure S2.** Water condensation time sequence during paper spray of pentane (a) ~30 seconds after spray was initiated; (b) to (d) taken consecutively from 60 seconds to 2 min after spray initiation.

#### 4. Examples of non-polar solvent paper spray ionization of drugs, peptides and lipids

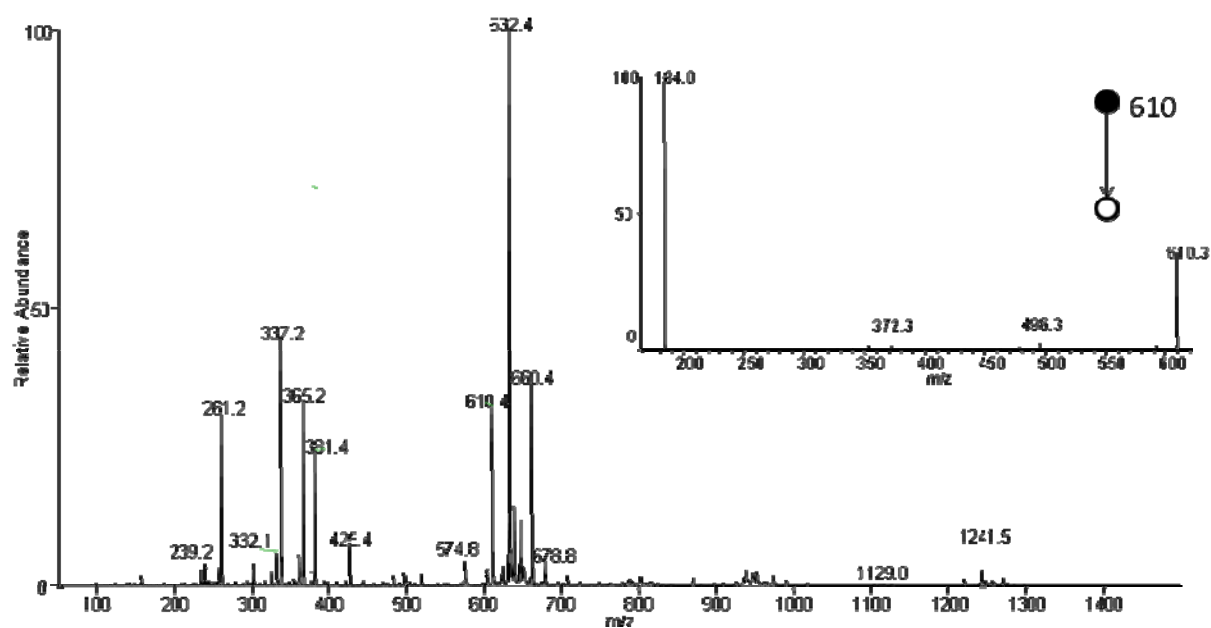
This section shows examples of paper spray analysis of various polar compounds using n-hexane as solvent. The analytes were examined as the solids from paper in either the positive or negative ion modes. Included are the pharmaceutical drugs, Ibuprofen, Verapamil, Amitriptyline and Doxorubicin, (Figure S3) the peptides angiotensin III and Phe-Gly-Gly-Phe (Figure S4), and 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (Figure S5). Tandem mass spectrometry (MS/MS) was used to confirm identifications. Only in the case of Doxorubicin are characteristic ions not observed and this is believed to be due to the limited mobility of this analyte in the alkane solvent as a result of its low hydrophobicity. Note that there is no delay in the appearance of the signal when switching the voltage from positive to negative modes, or vice versa, suggesting that the electric field does not play an important role in the transport of analytes in non-polar paper spray ionization.



**Figure S3.** Paper spray ionization (using hexane as solvent) for the analysis of pharmaceutical drugs deposited on the paper substrate, a) ibuprofen, 150 ng deposited, ionized in negative mode -2 kV with  $(M-H)^-$  as the base peak; b) Verapamil, 500 ng deposited, ionized in positive mode +2 kV with  $(M+H)^+$  as the base peak; c) Amitriptyline, 500 ng deposited, ionized in positive mode +2 kV with  $(M+H)^+$  as the base peak; d) Doxorubicin, 500 ng deposited on paper and ionized under the same conditions as in b) and c), no peak corresponding to the  $(M+H)^+$  was observed



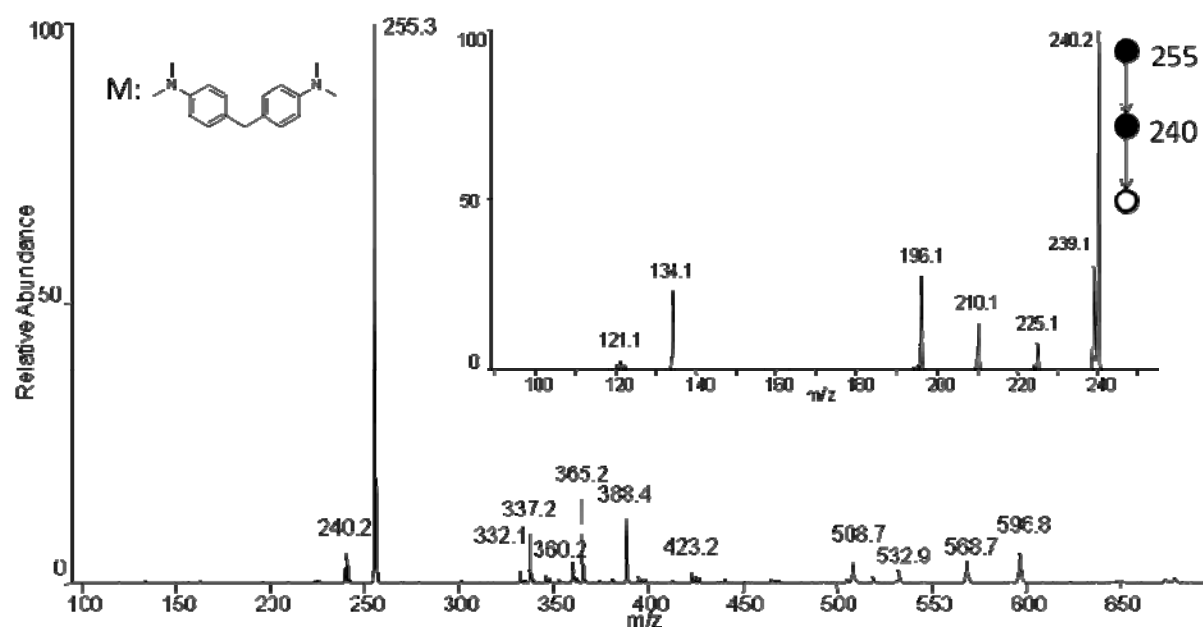
**Figure S4.** (a). Sequence specific fragments of the of the (M+2H)<sup>+</sup> ion (m/z 466) of Angiotensin III peptide (500 ng) obtained by MS/MS (corresponding full scan spectrum is shown in Fig. 2 (a)), using 5  $\mu$ L of a solution of 100 $\mu$ g/mL, methanol/water/HCl(1M), 49:49:2 v/v/v, and a potential of +1.8 kV with n-hexane solvent. (b). Peptide Phe-Gly-Gly-Phe, 1  $\mu$ g deposited on paper and allowed to dry; then ionized with hexane as spray solvent with a potential of +1.8 kV applied to the paper triangle. The mass spectrum showed m/z 427 (M+H)<sup>+</sup> and 853 (2M+H)<sup>+</sup> ions with small amounts of m/z 449 (M+Na)<sup>+</sup> ions. Inset is the and MS/MS spectrum of the (M+H)<sup>+</sup> ion.



**Figure S5.** Positive ion mode n-hexane paper spray (+1.8 kV) of 1-palmitoyl-2-glutaryl-sn-glycero-3-phosphocholine (500 ng) deposited on paper, this spectrum and that in Fig 2 were obtained using the same sample on a paper triangle simply by switching the voltage applied to the paper.

## 5. Paper spray ionization of solution-phase analytes

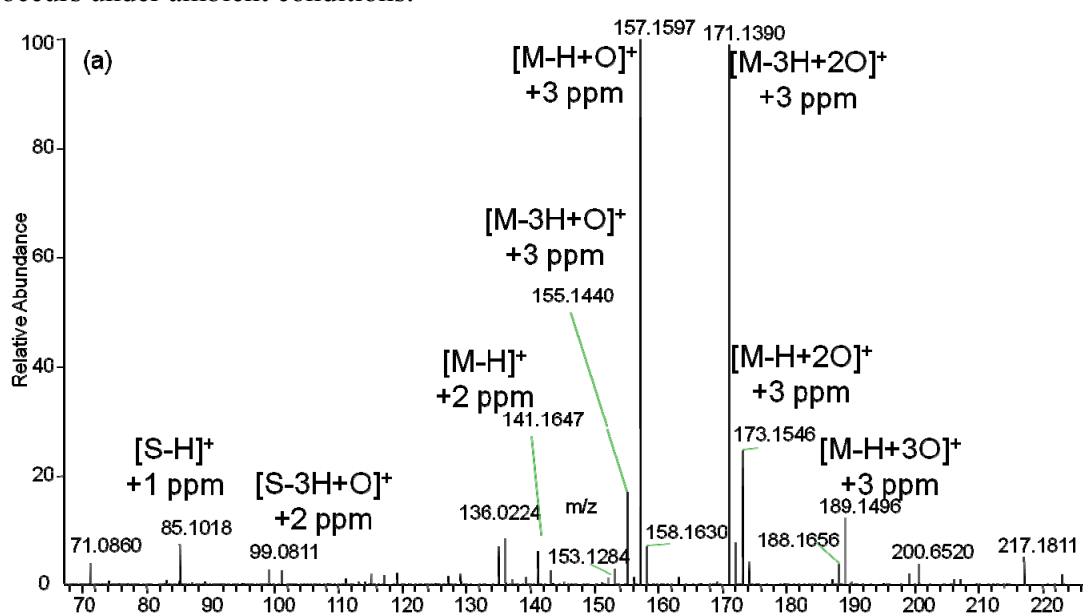
Analytes were examined from solution in this experiment, rather than as the solid as in the cases discussed so far. The analyte was deposited continuously onto the paper surface. This experiment allows ionization of analyte solutions that cannot be analyzed by ESI. Note that these particular solutions could not be ionized by either ESSI or nano ESI, even using applied voltages up to +6 kV. This is one demonstration that samples dissolved in non-polar solvent can be ionized directly from the solutions under ambient conditions, with the assistance of a paper triangle and voltage. This sampling method differs from those used so far in polar-solvent paper spray experiments and might be valuable in allowing real time on-line monitoring whether from non-polar or polar solutions.



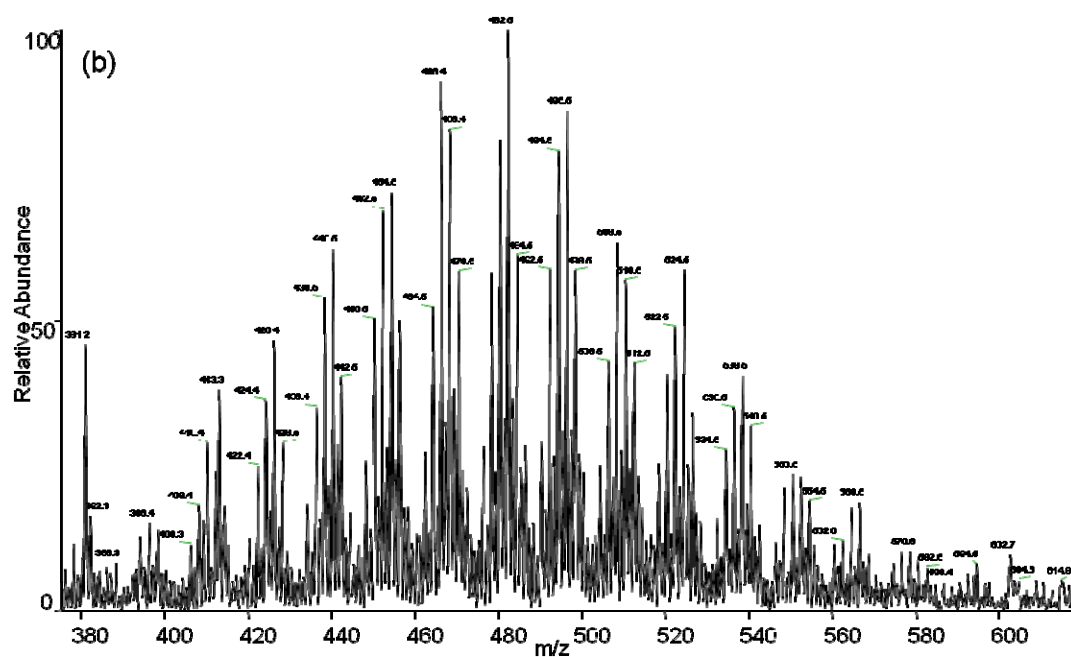
**Figure S6.** Solution of 4,4'-methylene-bis(N,N-dimethylaniline) in n-hexane solvent (1mg/mL) continuously fed to the +2 kV paper triangle yielded the protonated molecule  $(M+H)^+$ . The solution was supplied continuously to the paper. The protonated molecule was observed when the high voltage was applied. The inset is the MS<sup>3</sup> spectrum showing the fragmentation of  $(M+H)^+$  by loss of a methyl radical and displaying the fragments arising from this odd-electron product ion.

## 6. Paper spray ionization with non-polar solvents using high voltage conditions

This section describes a third type of experiment that uses non-polar solvent paper spray ionization. The three experiments taken in order are (i) ionization of solid, polar analytes using non-polar solvents (ii) ionization of solutions in non-polar solvents and this experiment, (iii) ionization of non-polar analytes like hydrocarbons. The experiment employs much higher voltages than those described so far. One example is the ionization of decane and hydrocarbon pump oil dissolved in hexane. Peaks corresponding to the distribution of the hydrocarbons were observed when the discharge occurred. The pump oil is non-volatile; paper spray of the non-polar solvent helps transport these non-volatile samples into the gas phase where ionization occurs under ambient conditions.



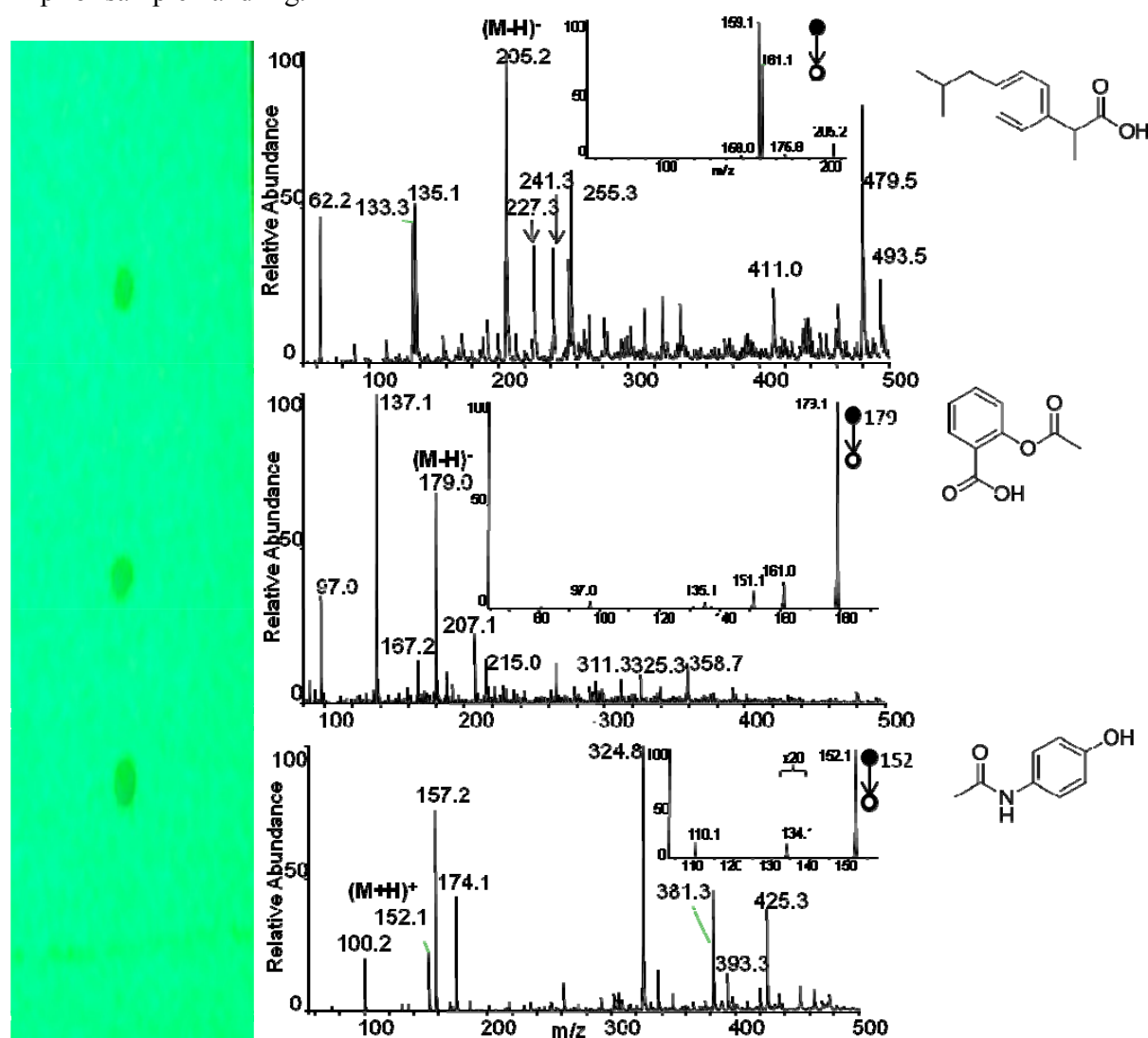




**Figure S7** Paper spray ionization with high voltage discharge, **(a)**. Decane dissolved in hexane (1:100) was fed to the paper triangle, a voltage between 2.5 kV and 7 kV was used to generate a discharge (characterized by greatly increased currents of  $\sim 1 \mu\text{A}$ ). Under these circumstances,  $[\text{M-H}]^+$ ,  $[\text{M-H}+\text{nO}]^+$ ,  $[\text{M-3H}+\text{nO}]^+$  types of ions for both the solvent ( $\text{S} = \text{hexane}$ ) and the analyte ( $\text{M} = \text{decane}$ ) can be observed. The identities of these ions were confirmed by exact mass measurements made using an Orbitrap mass spectrometer with the indicated mass accuracy. **(b)**. A mixture hydrocarbon sample (Inland # 19 pump oil, CAS#: 64742-65-0) 1000ppbv in hexane, was ionized by paper spray under conditions of high voltage discharge.

## 7. Paper spray ionization after TLC separation

Paper spray ionization with non-polar solvents, like the corresponding polar solvent experiment, can be performed in conjunction with planar chromatography. For example a drug mixture was separated by TLC using ethyl acetate solvent. Then the spot material was physically transferred to the paper substrate and analyte identities determined by paper spray ionization. Assignments were verified by MS/MS. In this example, the solvent used for ionization is different to that used in prior sample handling.

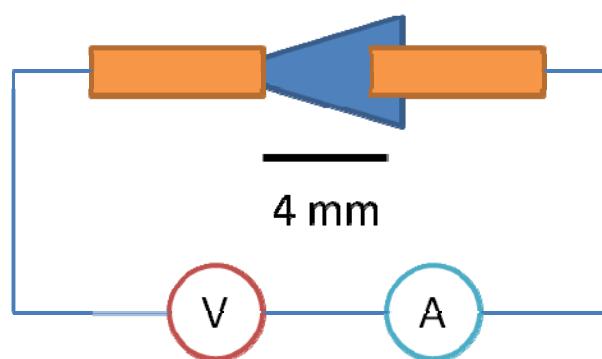


**Figure S8.** Three pharmaceutical drugs (ibuprofen, aspirin and acetaminophen) were dissolved in MeOH (1  $\mu$ g/mL) and spotted on a silica TLC plate. They were separated upon developing the TLC plate using ethyl acetate solvent. When the three silica spots were scratched off and put on a paper triangle. Continuous monitoring mode paper spray allowed the identities of the separated drugs to be verified by MS/MS.

## **8. Resistance measurements of the paper triangle under various conditions**

The resistance of the paper was measured under ambient conditions to obtain information relevant to questions of ionization and analyte transport. The experiment was done using the set-up illustrated in Figure S9. Under ambient conditions, the resistance of the paper was found to be in the range of  $3 \times 10^{12} \Omega$ . The presence of trace amounts of water is important for maintaining the conductivity of the paper substrate. When the paper was completely dried in a dry  $N_2$  environment, the resistance of the paper exceeded the measurement limit ( $4 \times 10^{13} \Omega$ ). In such an environment, paper spray ionization with hexane is not successful. Non-conductive paper substrates do not generate a high enough electric field at the tip to generate a spray. For the same reason, paper spray of non-polar solvents is also not successful if PTFE membranes are used, even though they work well if with polar solvents (such as iso-propanol).

When the paper was wetted with hexane under ambient condition, the resistance of the paper decreased 3- to 10-fold. This increased ionic mobility supports the transport mechanism suggested in the manuscript. It also supports the assumption that the electric field gradient is strongest at the paper tip and assists in field evaporation of the solvent.



**Figure S9.** The conductivity of the paper triangle (blue color in the figure) was measured by connecting it between two electrodes (gold color in the figure), varying the voltage and recording the currents. A larger piece of paper triangle (size: 1 cm high, 5 mm wide) was used for the convenience of handling.

## 9. Summary of paper spray solvents and comparison with nanospray

The Table S1 contains a short incomplete summary of solvents that can be used successfully in paper spray ionization. It includes both polar and non-polar solvents. Either can be used to analyze polar organic compounds. It is interesting to note that nano-ESI can be used to successfully to ionize polar analytes only when polar solvents are used, as judged by dipole moments and dielectric constants (yellow highlight). By contrast, polar insoluble compounds can be ionized in paper spray by all the solvents in the Table. Another interesting phenomenon is that two immiscible solvents can also be sprayed together in the paper spray experiment.

Table S1 Some solvents used in paper spray compared to nanospray

Solvent	Dielectric constant	Dipole moment	Viscosity	Surface Tension	b.p.	Paper spray	NanoESI
Water	78.3	1.84	0.89	72.0	100	Y	Y
Methanol	32.6	1.6	0.54	22.1	65	Y	Y
Ethanol	24.5	1.7	1.07	22.0	79	Y	Y
iso-Propanol	19.9	1.66	2.07	20.9	83	Y	Y
1-Butanol	17.5	1.66	2.5	24.9	117	Y	Y
n-Octanol	10.3	1.69	6	27.1	194	Y	Y
1,4-Dioxane	2.3	0.45	1.37	34.5	101	Y	Y
Toluene	2.4	0.36	0.59	28.5	111	Y	N
n-Pentane	1.84	0	0.24	15.5	36	Y	N
n-Hexane	1.88	0	0.29	17.9	68	Y	N
n-Heptane	1.9	0	0.39	20.3	98	Y	N
n-Decane	2.0	0	0.92	23.4	175	Y	N

### References:

1. H. Wang, J. Liu, R. Cooks and Z. Ouyang, *Angewandte Chemie*, 2010, **122**, 889-892.