Measurement of Nitrophenols in Rain and Air by Two-dimensional Liquid Chromatography – Chemically Active Liquid Core Waveguide Spectrometry.

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SUPPORTING INFORMATION

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Reagents and Materials

4-Nitrophenol, 2-Methyl-4-Nitrophenol, Amberlite® XAD-2 (20-60 mesh), Amberlite® XAD-4 (20-60 mesh), Amberlite® XAD16 (20-60 mesh), Silica gel (Davisil®, Grade 636, pore size 60 Å, 35-60 mesh, Product No. 236802) were purchased from www.sial.com. 5-Methoxy-2-Nitrophenol (Product No. 335440010) was from www.acros.com. 2-Nitrophenol, 2,4-Dinitrophenol, 2-Methyl-4-Nitrophenol, Methanol (HPLC grade), sodium sulfate, ammonium acetate and Ammonium hydroxide were obtained from www.vwrsp.com.

All solutions were prepared using milli-Q water (18.2 MΩ resistivity, Millipore®, Billerica, MA).

Standard Gaseous Nitrophenol Calibration Source. We made permeation tubes filled with solid NPs (2-NP, 5-MeO-2-NP, 4-NP, 2,4-DNP). PTFE tubes, even with relatively thin walls allowed very little permeation of NPs. Low density thin wall polyethylene (LDPE) tubes (0.062 in. I.D. x 0.010 in. wall x 2.56 in long, www.scicominc.com) were used. The permeation tubes were maintained at a constant temperature of 30±0.1°C and calibrated gravimetrically. Even with these thin-walled LDPE tubes, the permeation rates of 4-NP and 2,4-DNP were too small to measure (at 30 °C, 4-NP has ~1/400th the vapor pressure of 2-NP). The permeation tube output was measured as 25.9 ± 0.3 and 78.8± 1.3 ng/min for 2-NP and 5-MeO-2-NP (these values tallied well with serial bubbler collection of permeation tube outputs and quantitation by UV-vis absorption)and was diluted and humidified (as desired) in a 2-stage system using pure air from a pure air generator (www.aadcoinst.com) and various mass flow controllers (See Figures S1 and S2 overleaf).

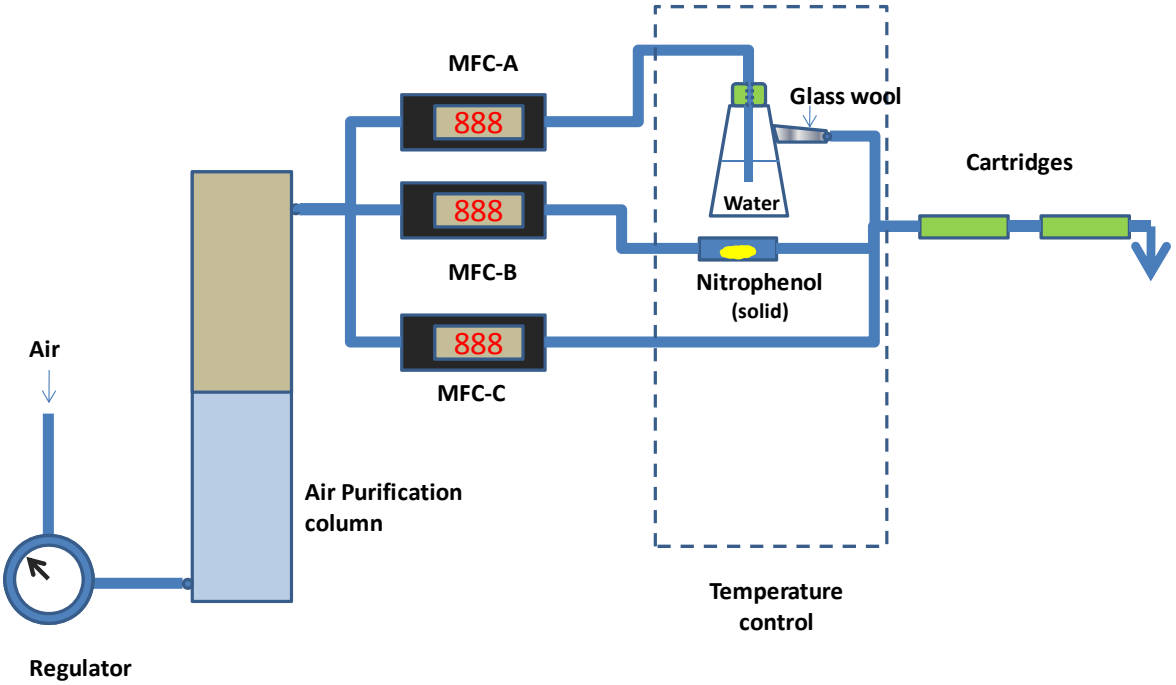

Figure S1. Permeation tube arrangement. MFC B typically maintained at 0.1 L/min. Serial cartridge test arrangement shown. The total flow rate through cartridge is A+B+C, humidity is varied by changing MFC-A to MFC-C flow ratio.

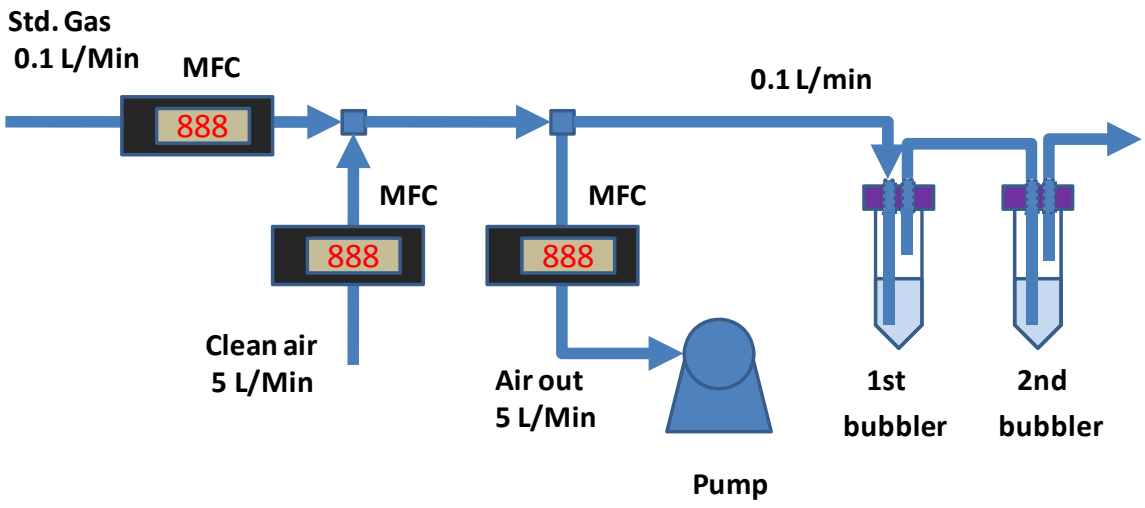

Figure S2. Experimental setup for the wet determination of nitrophenols, calibrating the standard gas source. Standard gaseous nitrophenols were diluted with purified air and were subsequently trapped in dilute base. The resulting solution was measured by direct spectrophotometry or by HPLC – LCW absorbance detection. Sampling time: 10 min.

Chemically Active Liquid Core Waveguide (CA-LCW) Detector. The system in Figure 2 is described in more detail here. A Teflon® AF capillary (150 μm i.d., 0.8 mm o.d., 1.42 m long), used as a degasser tube in electrodialytic eluent generators (Dionex) was salvaged from a used unit. A 5 mm light emitting diode (5UV395-30, www.etgtech.com, measured λ_{max} 403 nm) was cut flat and polished, with the emitter chip just below the polished surface. A 0.15 mm dia. hole was drilled at an angle from the side of the LED to a location just above the emitter chip. The initial ~1 mm of the hole was enlarged with a 0.375 mm dia. drill bit and a 0.175 mm i.d. x 0.375 mm o.d. tube was forcibly inserted into this and epoxied in place. The AF capillary was forcibly inserted inside a PEEK tube segment (0.030-in I.D. x 0.062-in O.D.) that in turn was epoxied into a stainless steel tube segment (0.063-in I.D. x 0.083-in O.D., HTX-14-36, www.smallparts.com). This tube assembly was placed on the polished flat surface of the LED just above the drilled hole, clamped and epoxied in place using high strength extended-cure epoxy. The other end of the AF tube was prepared similarly as the first except that the outer sleeve was a PEEK tube ($^1/_{16}$ in i.d., $^1/_8$ in. o.d.), rather than a stainless steel tube. This end of the assembly was also epoxied on the hemispherical ball lens of a light-to-voltage converter, a monolithic photodiode-operational amplifier combination (TSL252LF, www.taosinc.com) using 24 hour curing epoxy adhesive. As shown in Figure 1 inset, solution outlet was provided by drilling a hole through the external PEEK jacket. The LED was powered at 4.1 mA (390 Ω series resistor, 5 V applied). The light throughput efficiency of the LCW can be judged from the fact that this relatively small drive current was sufficient to provide nearly full output from the detector. The TSL photodetector output was filtered with a RC filter of 1 s time constant. The detector output is in terms of transmittance. With a scale factor of baseline absorbance as 100%T, absorbance (A) was calculated as $A = -\log_{10} T$ by software.

All but 10 cm at each end of the AF tube was put within a 50-mL capacity polypropylene jar containing ~25 mL concentrated ammonia at the bottom diluted

1:1 with water. In later experiments concentrated ammonia was used without dilution and a rubber stoppered bottle was used (this is the arrangement shown in Figure 2). The entrance and exit points of the AF tube into the jar were sealed with hot-melt adhesive. Ammonia permeates through the highly permeable AF tube making it a chemically active waveguide and pH of the internal solution is raised.

Acetonitrile and methanol are often introduced to purely aqueous eluents prior to ESI/MS for improving ESI efficiency. With thin-walled Teflon AF tubes, significant amounts of ACN or MeOH can be introduced in this manner without the need of an additional pumping system.

Tandem Mass Spectrometry. Fragmentation and monitoring details for specific analytes are given here. Selected ion monitoring mode after fragmentation, controlled by Xcalibur version 2.0, utilized a collision voltage of 16 V for 2- and 4-NP and monitored the transition $O_2NPhO^- \rightarrow$ (-NO) $\rightarrow OPhO^-$ (138.1 Th $\rightarrow$ 108.1 Th). For methyl nitrophenols, the collision voltage was 15 V and monitored the transition $O_2N(Me)PhO^- \rightarrow$ (-NO) $\rightarrow O(Me)PhO^-$ (152.1 Th $\rightarrow$ 122.1 Th). For 2,4-DNP, the collision voltage was 24 V and we monitored the transition $(O_2N)_2PhO^- \rightarrow$ (-CO,-NO_2) $\rightarrow C_5H_3NO_2^-$ (183.0 Th $\rightarrow$ 109.4 Th)[2].

Relationship Between pH and Absorbance for Specific Analytes.

Consider that a given NP in unionized form (HIn) has a molar absorptivity of ε_{HIn} and in ionized form (In^-) this is ε_{In} at the monitoring wavelength. If the acid dissociation constant of the specific NP is K_{In} and the two different pH levels (pH_{fin} and pH_{in}) at which the absorbance is monitored corresponds to hydrogen concentrations of H_{fin} and H_{in}, the absorbance at each pH will be given by

$$A_{fin} = C(K_{In}\varepsilon_{In} + H_{fin}\varepsilon_{HIn})/(K_{In} + H_{fin}) \quad \ldots(1)$$

$$A_{in} = C(K_{In}\varepsilon_{In} + H_{in}\varepsilon_{HIn})/(K_{In} + H_{in}) \quad \ldots(2)$$

The absorbance ratio R is independent of the concentration C and as long as H_{fin} and H_{in} are fixed, will be governed by the indicator-specific parameters ε_{HIn}, ε_{In} and K_{In}.

[2] Thomson (Th) is the new unit for m/z

For the common situation where ε_{HIn} is essentially zero at the monitoring wavelength, a considerably simplified form of the signal ratio R emerges:

$$R = A_{fin}/A_{in} = (K_{In} + H_{in})/(K_{In} + H_{fin}) \quad \ldots(3)$$

It is notable that this ratio is independent of ε_{In} and If H_{fin} and H_{in} are known, K_{In}, an independent characteristic of each NP analyte, can be readily evaluated.

Dependence of Response on CA-LCW Length.

Intuitively, cell volume increases with cell length and dispersion will eventually deteriorate resolution. Obviously if the cell volume is so large that an entire peak is inside the cell at any given time, the absorbance due to that particular peak cannot be possibly any greater and for any given peak there must be a maximum length beyond which there is no further gain and only increased dispersion. Dispersion affects the resolution of adjacent peaks and a fixed volumetric dispersion induced by the measurement cell will obviously affect the sharpest peaks more than any other. In an isocratic elution system and assuming normal chromatographic behavior, this will be the first peak. This will have the minimum volume and will dictate what cell volume can be tolerated. Figure S3 overleaf shows an example, with overlaid chromatograms of 6 NPs with a 13 μL volume PDA detector and two CA-LCW detectors of 13 cm (4.1 μL) and 62 cm (19.5 μL) volume, respectively.

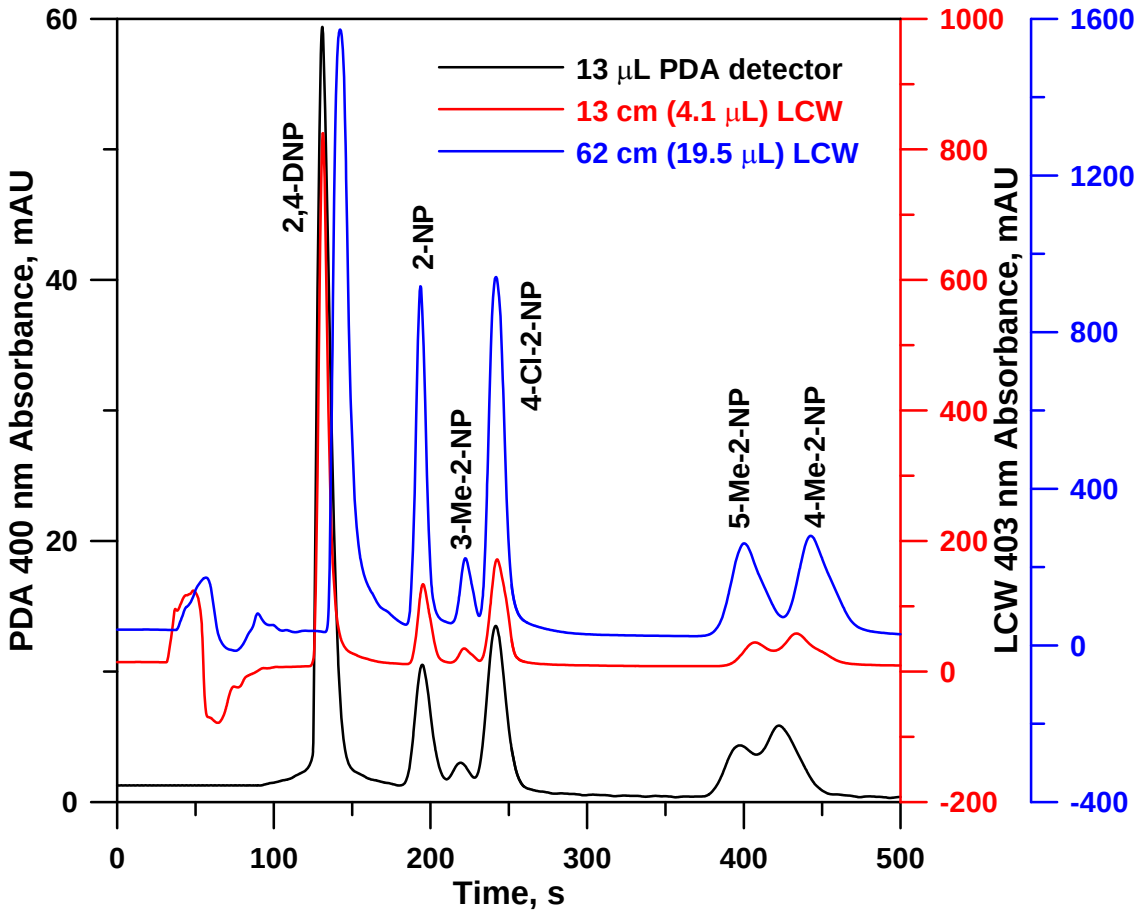

Figure S3. Isocratic elution of nitrophenols 100 µg/L each on 5 cm x 2 mm Acclaim 120 column. 0.5 mL sample preconcentrated on a AG21 anion exchanger precolumn. Eluent: 4 mM Ammonium acetate in 42% methanol, 0.25 ml/min. Black trace: Dionex PDA 100 photodiode array detector, cell volume 13 µL. Red trace: 13 cm CA-LCW detector, 4.1 µL volume, Blue trace: 62 cm CA-LCW detector, 19.5 µL volume. The numerical Figures of merit are given in Table S1 overleaf.

Table S1. Performance data for Chromatograms in Figure S3.

Peak Identity	PDA Half width sec	PDA Plates/m	PDA Resolution Pk 5 & 6	13 cm LCW Half width sec	13 cm LCW Plates/m	13 cm LCW Resolution Pk 5 & 6	62 cm LCW Half width sec	62 cm LCW Plates/m	62 cm LCW Resolution Pk 5 & 6
2,4-DNP	9.50	21040		6.32	47770		10.80	20045	
2-NP	11.12	34050		8.82	54300		7.16	80980	
3-Me-2-NP	9.87	54680		8.99	67290		8.29	79570	
4-Cl-2-NP	13.35	36340		11.92	45910		10.93	54240	
5-Me-2-NP	21.35	38440		18.32	54770		22.92	54240	
4-Me-2-NP	29.75	22310	0.57	25.58	31840	0.71	24.09	37420	1.07

It will be obvious from Figure S3 and Table S1 that while some of the behavior is readily understood (the PDA cell volume is too large for this column and there is across the board increase in efficiency in going from the PDA to the short LCW cell) other aspects of the observed behavior are not so readily apparent. Why peak efficiency (and resolution) increases as the LCW length is increased from 13 to 62 cm may be less apparent to the reader. This arises because only a limited amount of ammonia can be introduced through the short LCW – the pH is such that the pH induced ionization plateau has not been reached. Comparison of the peak heights between the 13 cm and the 62 cm detector will indicate that in all cases except for 2,4 DNP, the gain in going from the former to the later LCW is significantly more than the length factor of 62/13 $\underset{\sim}{}$ 4.75. For 2,4-DNP the absorbance is high enough for nonlinearity and stray light to play a role and the peak volume is also small. In both LCWs absorptivity rises as the NPs ionize across the length of the LCW, likely reaching a plateau for the longer LCW well before the end. The absorptivity increase and the optical path length increase are not additive but multiplicative. The absorbance thus does not rise simply by some factor but is raised to a power. Such power transformation has the consequence that plate counts and resolution actually improves.[3]

[3] Dasgupta, P. K.; Serrano, C. A.; Fairchild, J. N.; Shalliker, A. R.; Guiochon, G. *J. Chromatogr. A* (submitted)

Calibration Data.

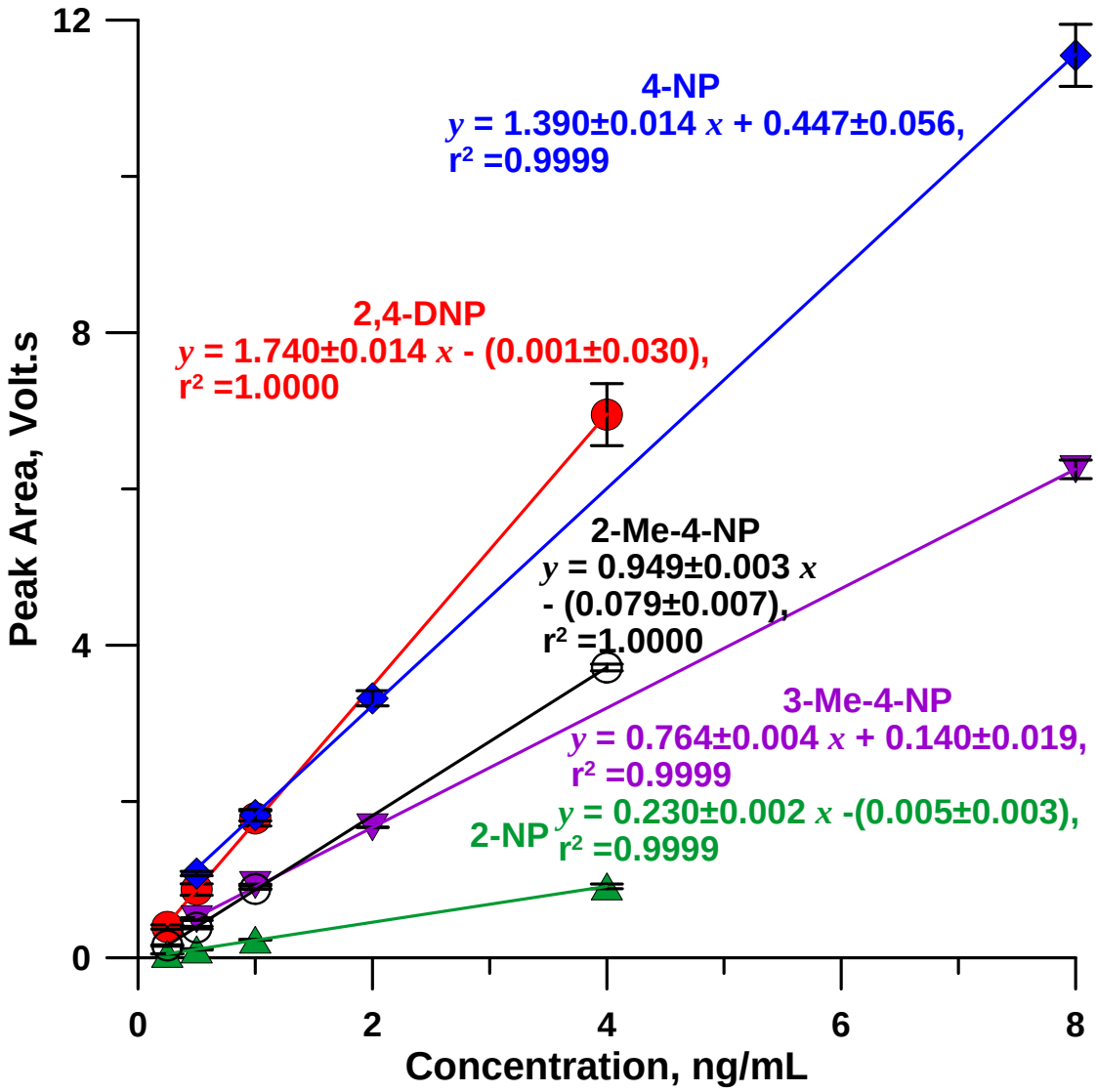

Figure S4. Calibration data for five common nitrophenols. CA-LCW detection at 403 nm.

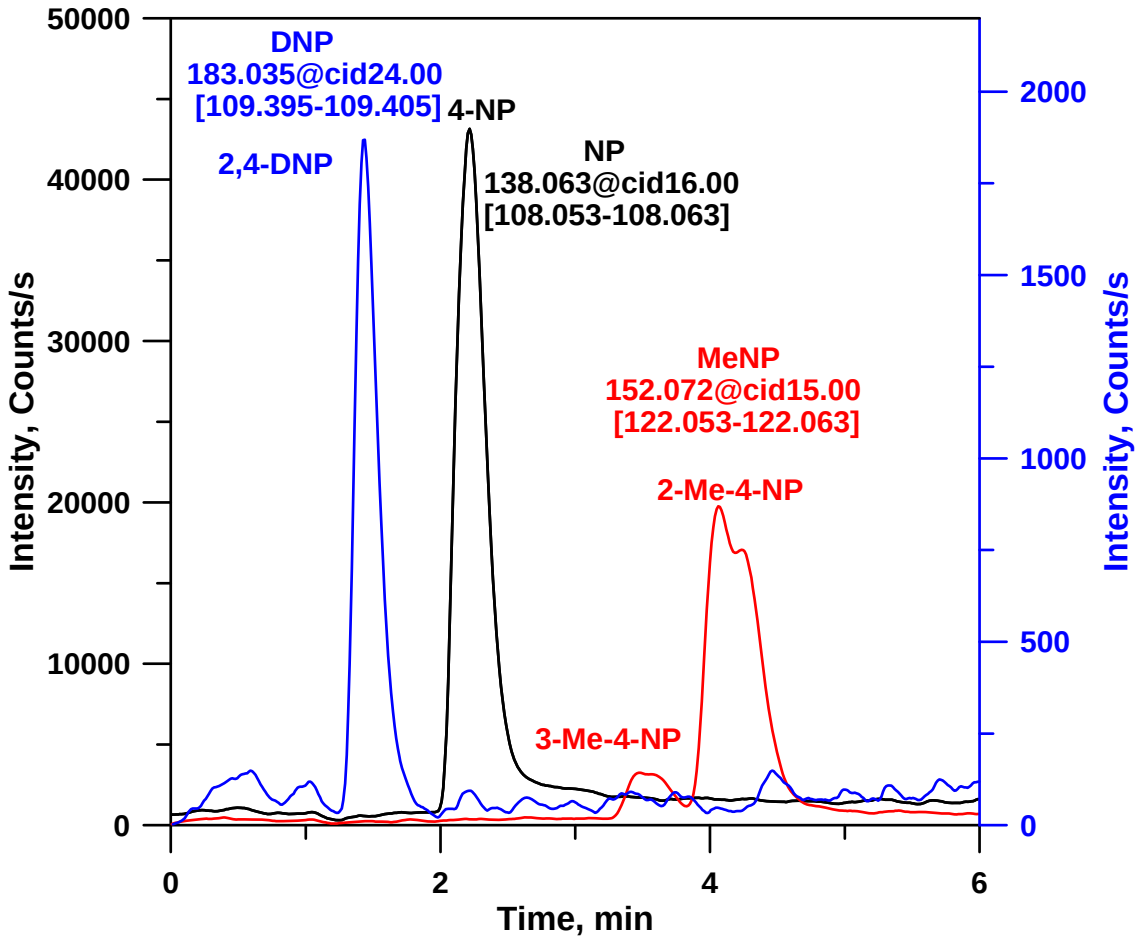

Figure S5. MS/MS chromatogram of the rain sample collected at the University of Texas at Arlington, TX, USA. The monitored ions had SRM m/z transitions of: 138.0 → 108.0 (4-NP 630 pg/mL), 152.0 → 122.0 (3-Me-4-NP, 56 pg/mL and 2-Me-4-NP, 178 pg/mL), and 183.0 → 109.0 (2,4-DNP, 97 pg/mL).

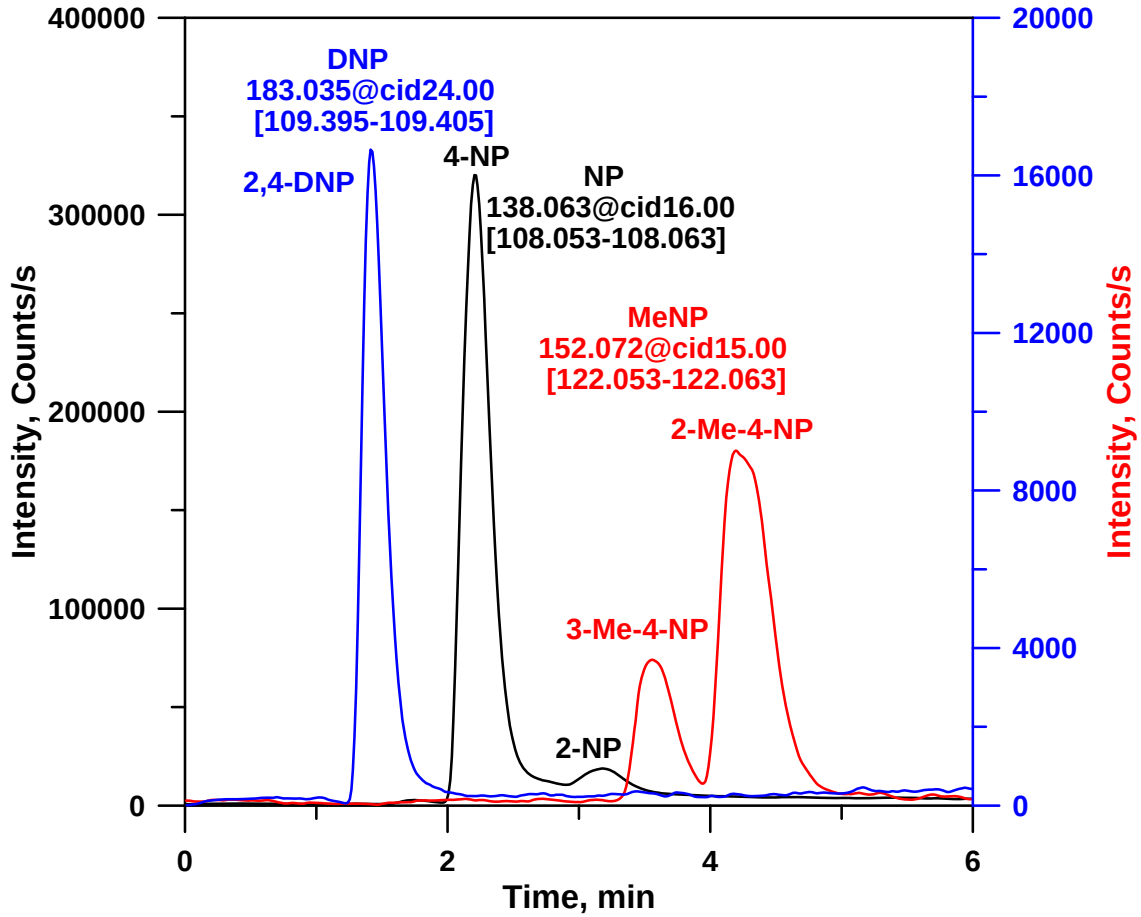

Figure S6. MS/MS chromatogram of the air sample collected at the University of Texas at Arlington, TX, USA. The monitored ions had SRM m/z transitions of: 138.0 → 108.0 (4-NP, 4.67 ng/mL in extract and 2-NP, 2.84 ng/mL), 152.0 → 122.0 (3-Me-4-NP, 68 pg/mL and 2-Me-4-NP, 83 pg/mL), and 183.0 → 109.0 (2,4-dinitrophenol (2,4-DNP, 870 pg/mL). An additional methyl nitrophenol at low levels eluted at 7.45 min, not shown here.

3-Methyl-2-Nitrophenol vs. 3-Methyl-4-Nitrophenol.

At first we separated the 3-methyl-2-nitrophenol / 3-methyl-4-nitrophenol isomers on a Cyclobond I 2000 column (4.6 mm x 25 cm x, 20024AST, www.sial.com). Using a 40:60 acetonitrile:water mobile phase, these isomers were readily separated with the 3-Me-2-NP eluting first and the 3-Me-4-NP eluting later (Figure S7):

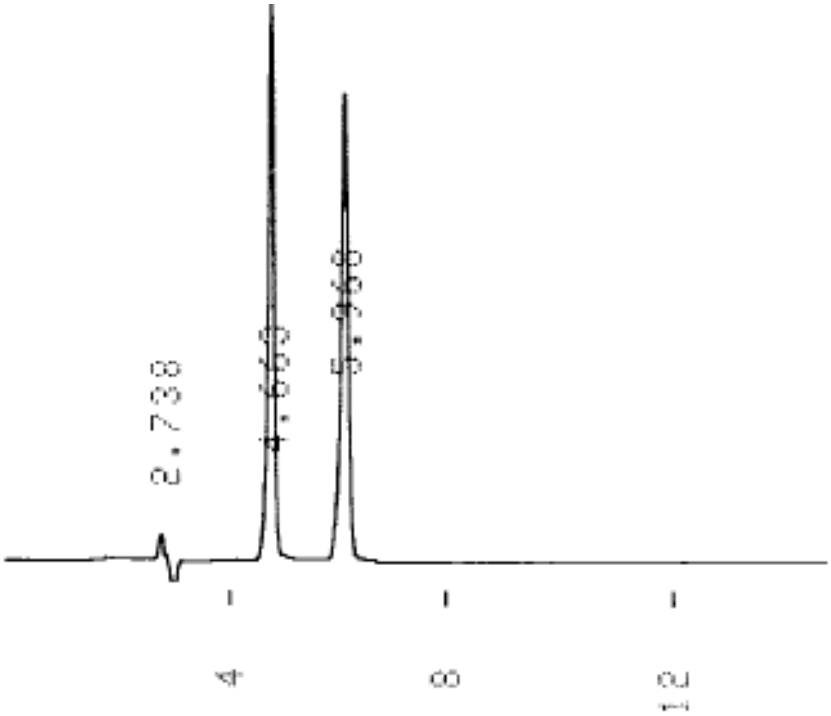

Figure S7. Separation of pure isomers UV detection at 254 nm.

The separation was next conducted on a similar smaller bore column (2.1 mm x 15 cm, 20019AST www.sial.com) with 100 µL injection 50:50 MeOH: H_2O elution at 0.20 mL/min and MS/MS detection. 3-Me-4-NP is detected with nearly 2 orders of magnitude better response than 3-Me-2-NP.

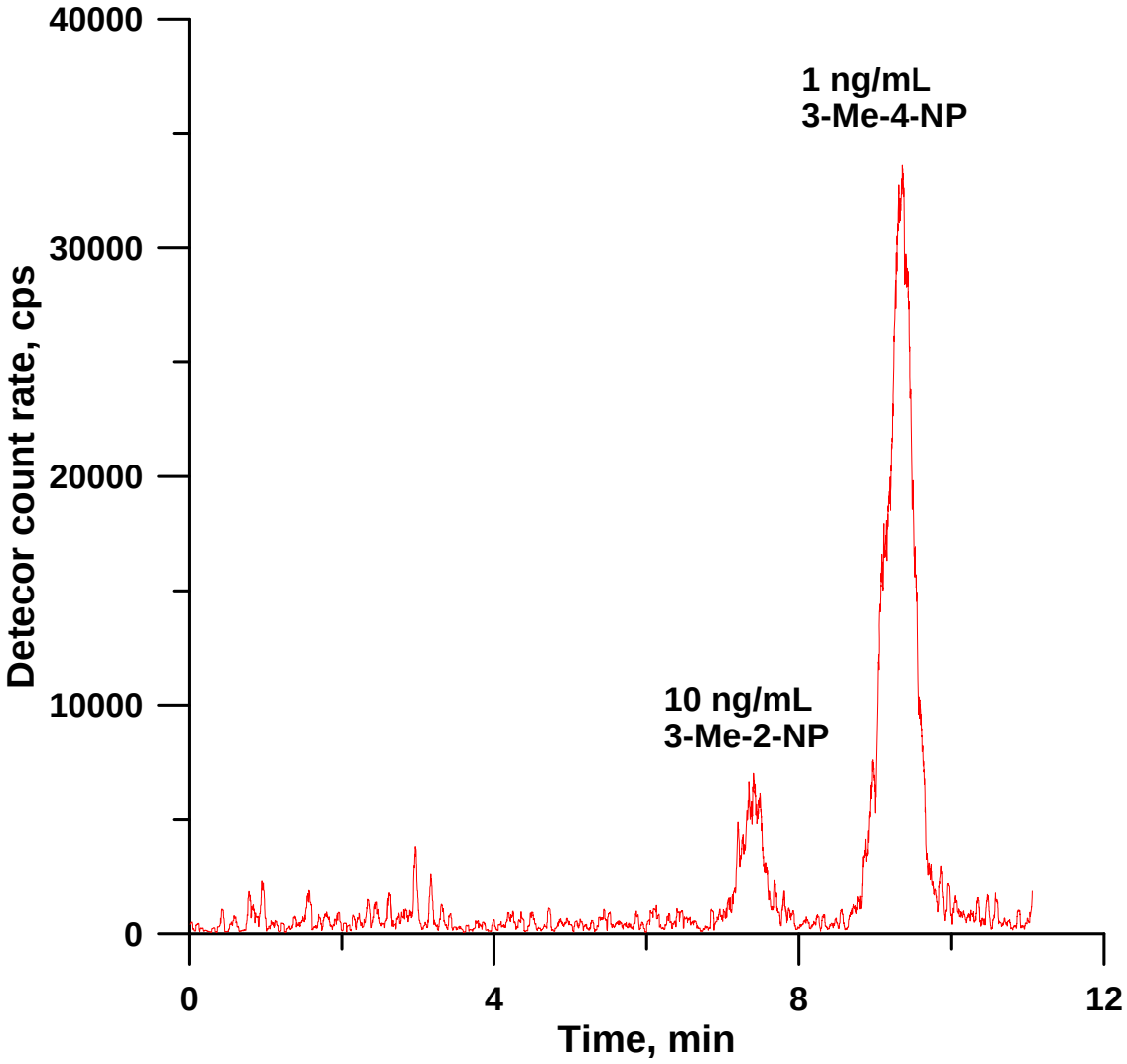

Figure S8. Selected fragmentation monitoring mode: 152.0 → 122.0 Th, NO is lost from 3-Me-NPs. Note that in terms of intensity 3-Me-4-NP is about 54 times more responsive than 3-Me-2-NP.

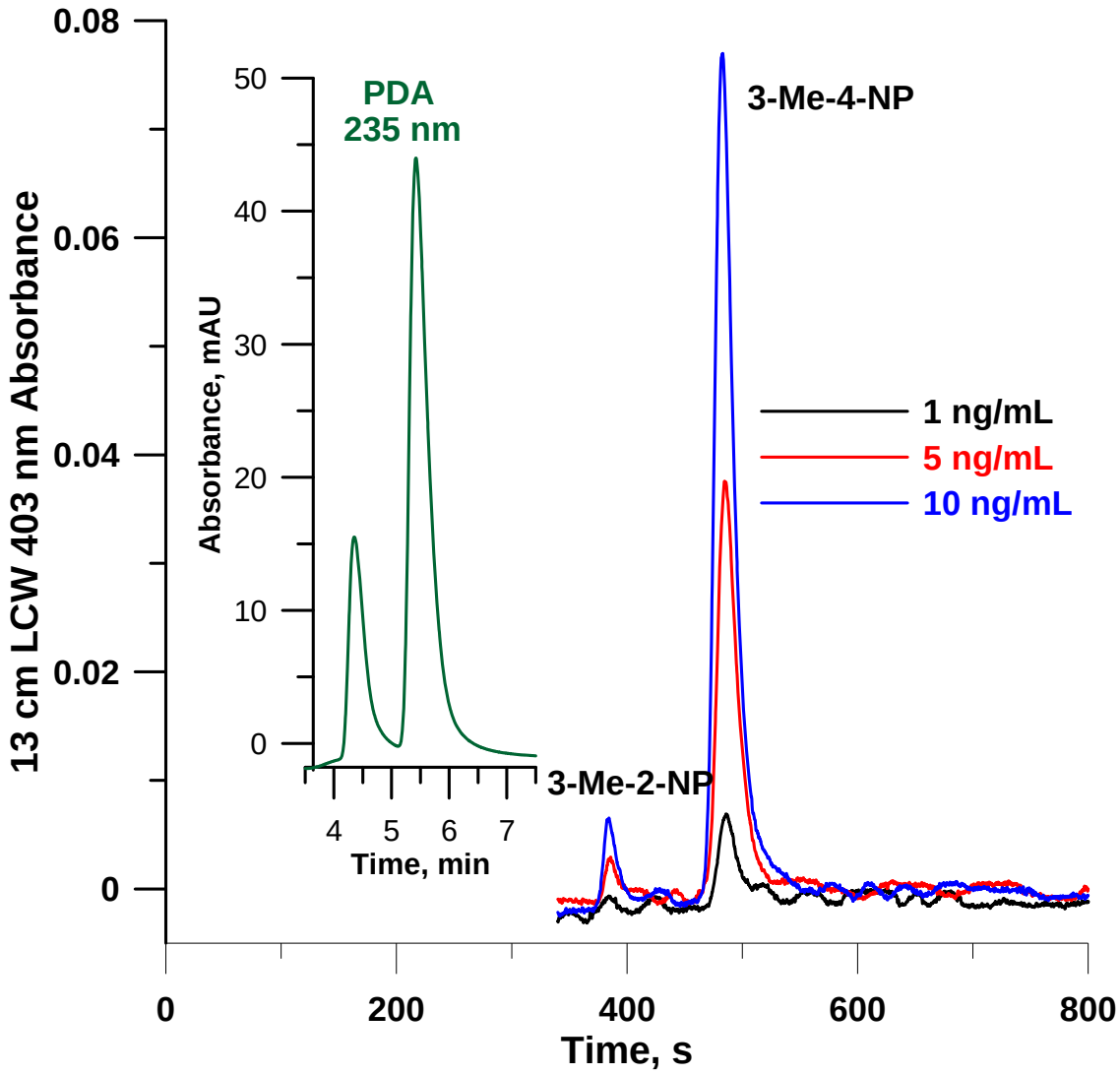

Figure S9. 3-Me-4-NP and 3-Me-2-NP are separated and detected with a short (13 cm) CA-LCW detector on an Supelco CYCLOBOND 2000 column (2.1 mm x 15 cm, 20019AST www.sial.com), 50:50 MeOH/water ,0.25 mL min^{-1}. 500 µL injection volume; there is initial disturbance from the large volume injection. The LCW was exposed to concentrated undiluted NH_3. Note that the response of 3-Me-2-NP is very much lower than 3-Me-4-NP, much like MS. The left inset shows the detection of 1 µg/mL each of the two isomers in a mixture and UV detection at 235 nm with a conventional Photodiode array detector (PDA-100, Dionex), note that in this case response of 3-Me-2-NP, although still ~2.5x lower than 3-Me-4-NP, is much more comparable.

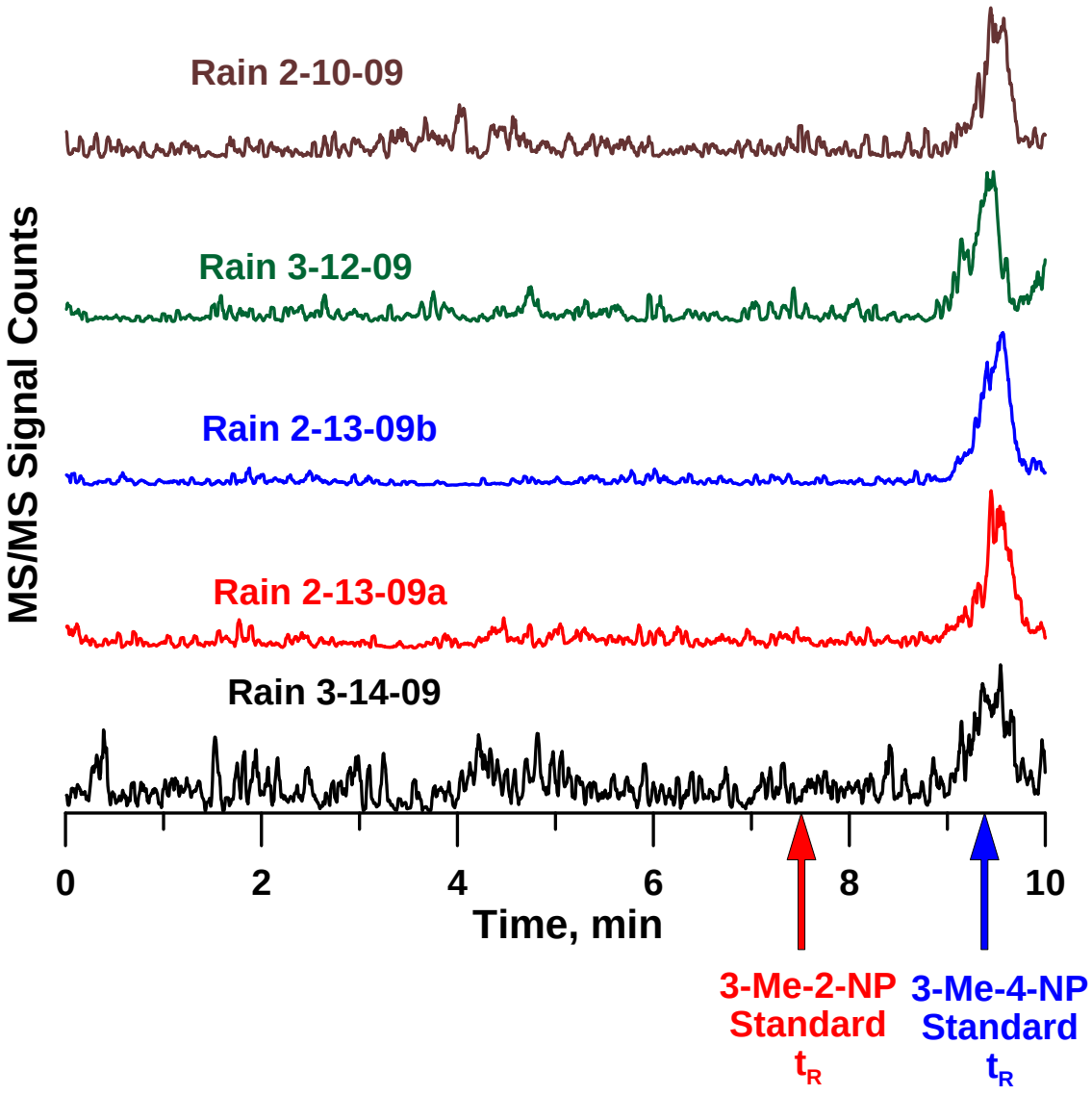

Figure S10. Houston rain samples analyzed for methyl nitrophenols. Selected fragmentation monitoring mode: 152.0 → 122.0 Th, NO is lost from Me-NPs. In virtually all samples another unknown Me-NP elutes at ~10.5 min, not shown here.

There are literature reports of the occurrence of 2,6-DNP as well, which in the present analytical protocol coelutes with 2,4-DNP. Here also the cyclodextrin-based stationary phase is able to easily separate the two.

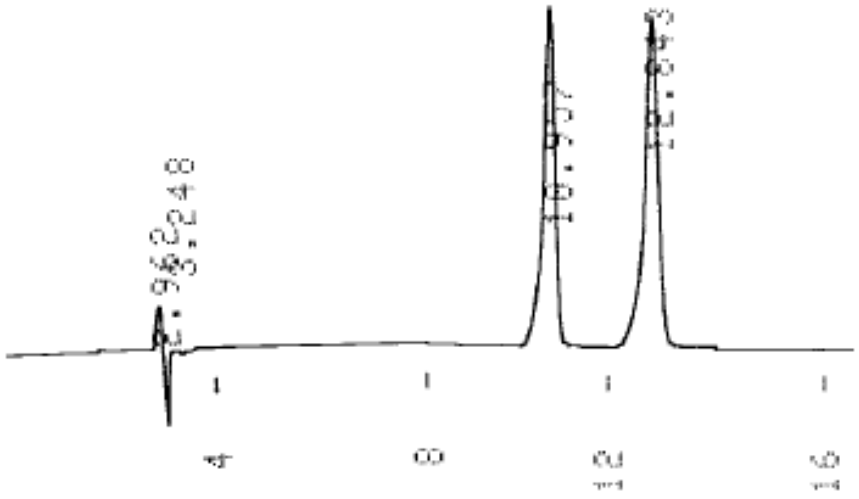

Figure S11. Separation of 2,6-DNP (eluting first) and 2,4-DNP on a Cyclobond I 2000 column 4.6 mm x 25 cm. Eluent 70:30 0.2%v/v HCOOH : acetonitrile.

Based on similar experiments as above, a limited number of rain samples tested contained almost exclusively 2,4-DNP and no 2,6-DNP.

Table S2. Recoveries of NPs depending on methanol content of solvent. Nitrophenolic species in air sampled cartridges were eluted by 25%, 50% and 100% methanol (2 mL) and adjusted to 4 mL with 18.2 MΩ milli-Q water. The solutions were filtered with 0.45 μm pore size nylon filter and were transferred into the vials of the auto-sampler to be directly preconcentrated on a preconcentrator column (AG 21, Dionex Corp.).

Nitrophenols	Injected (ppb)	Eluted by 25 % MeOH(ppb)	Eluted by 50 % MeOH(ppb	Eluted by 100 % MeOH(ppb)
2,4-DNP	2	2.04 ± 0.06	2.14 ± 0.03	nd
4-NP	2	2.02 ± 0.05	1.97 ± 0.10	nd
2-NP	2	2.00 ± 0.1	1.70 ± 0.30	nd
3-Me-4-NP	2	1.92 ± 0.03	1.70 ± 0.20	nd
2-Me-4-NP	2	1.89 ± 0.03	1.80 ± 0.10	nd