Electronic Supplementary Material (ESI) for Analyst
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Supporting Information

Direct Analysis of 4-Methylimidazole in Foods using Paper Spray Mass Spectrometry

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1. Experimental conditions

(a) Ion sources: Paper spray voltage: 2.5~3.5 kV; nanoESI voltage: 1.5 kV.

(b) Thermo Finnigan TSQ triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA) was used under the following instrumental conditions unless specified otherwise: capillary temperature: 300 °C; capillary Offset: 15 V; Q1 Peak Width: 0.7; Scan Width (Q3): 0.01; Scan Time 0.1 s; Skimmer Offset: 6; Q2 CID Gas: 1.5 mTorr (Argon);
MRM Conditions:
m/z 83 → 56: tube-lens voltage 81v, collision energy 21 (mnfg. arbitrary units)
m/z 86 → 59: tube-lens voltage 80v, collision energy 17 (ditto)

(c) Thermo Finnigan LTQ linear ion trap (Thermo Scientific, San Jose, CA) was used under the following instrumental conditions unless specified otherwise: capillary temperature: 150 °C; Capillary voltage: 15 V; Tube lens: 65 V;
MRM Conditions: isolation window *m/z* 1.5, q_z value 0.5; activation energy 45%;
Ion trap Simultaneous MRM Conditions: isolation window *m/z* 81.5 to 87.5, q_z value 0.5; activation energy 45%;

2. Detailed quality control protocols

A. Identification of the analyte was confirmed 1) in the full scan fast screening mode by meeting the requirement that exact mass measurements of both the $^{12}C/^{13}C$ isotopic peaks be within <0.0005 amu of the correct value and the isotopic ratio 13C/12C) ratio be within 35% of the theoretical value; 2) in qualitative and quantitative tandem mass modes, MRM transitions (unit mass resolution for precursor and product) must match those of standard compounds, and in quantitative analysis, their ratios must be within ± 30% of the mean values for the standard compounds.

B. To check for and avoid carry-over, a blank-run was carried out in the beginning of each paper spray experiment run. The same experiment conditions (elution solvent, voltage etc.) and identification requirements were used. Clean-up of mass spectrometer's inlet capillary and other equipment was performed if carry-over was identified.

C. Each batch run consisted of a minimum of a five-point calibration with concentrations bracketing the expected sample concentration. Multiple batch runs (some repeated on different work days) were required to yield >3 data values for each sample and each calibration point. These data were grouped to provide relative standard deviations. Data outliers, if present, were not deleted. The deviation of the results from (>3) repeating batch runs was evaluated and to be acceptable, the CV had to be ≤ 25%. If higher, the analyst investigated the situation and took remedial action.

D. Prepared mid-level standards (QC samples) were analyzed at ~10 unknown sample intervals when a series of unknown samples was being analyzed. The acceptance error was within ± 20% of the result of the first reference standard.

E. When a sample analysis result was found to fall outside the range of calibration curve, the sample was diluted and reanalyzed.

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F. The performance of each batch run of the calibration was evaluated by the linearity (R^2>0.98) of the calibration curve and the error of the repeated reference standards (within ± 20% of the known value). If the performance was not within the required range, the instrument was inspected for malfunctions and corrective actions taken. Reanalysis of impacted samples was then performed.

3. Determination of detection limits

The detection limit (**DL**) was defined as the concentration that produces a signal higher than 3 times of standard deviation plus the mean value of the blank runs. The instrument detection limit **(IDL)** is determined by analyzing low concentrations of *neat standard sample*. On the other hand, the limit of detection (**LoD**) was determined by analyzing low concentrations of samples spiked *into the target matrix*. The limit of quantitation **(LoQ)** was determined as the lower end in the investigation of the practical linear range of quantitation. LoQ was defined as the concentration that results in a signal 20 times of standard deviation plus the mean value of the blank runs. Figure S1 illustrates one example of the determination of background signal level for IDL and LoD. Based on the blank signal level, detection limits were obtained by analyzing low concentrations of sample solutions, as shown in Figure S2.

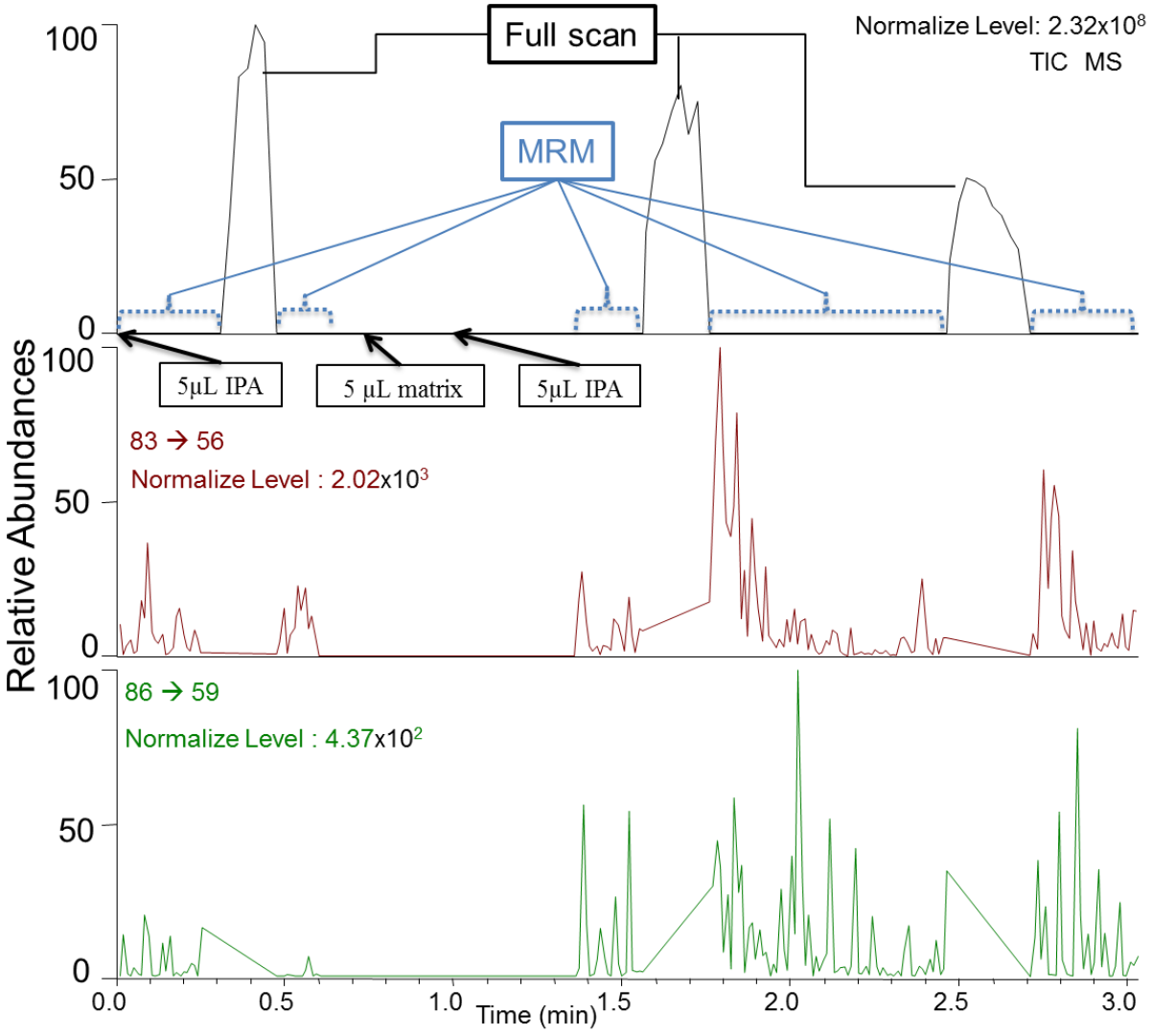

Figure S1. Noise level used in determination of IDL and LOD. MRM transitions were monitored while analyzing neat solvent and blank matrix (S 006 1/100). Full scan mass spectra were also recorded several times to make sure that low MRM signals were not the result of poor spray performance.

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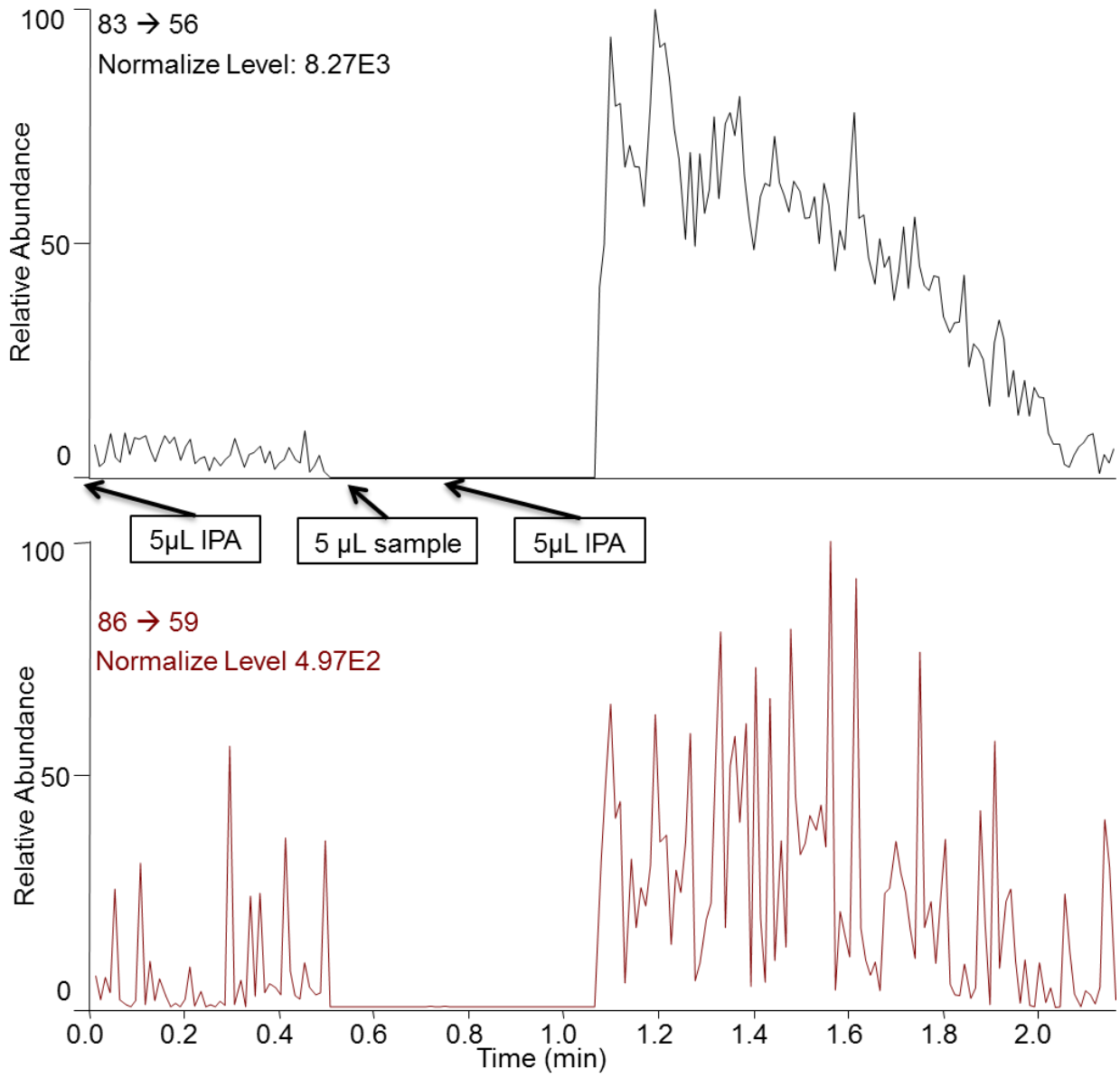

Figure S2. Signal response of 4-MEI solution (5 ppb in blank matrix). The MRM transition for the D_3-4-MEI was also recorded for comparison, although no internal standard was spiked into this sample.

The detection limits were first estimated and then verified by running 20 repeats at the estimated level of the unknown. As shown in Table 1, the LoD was determined to be 5 pg/µL. Similarly, the IDL was determined to be 3 pg/µL. At concentrations between 5 and 10 pg/µL, the response signal readily surpassed 20 times of noise level. However, for a practical LOQ value in the case of running samples spiked with internal standard, the standard deviation of sample runs must also be taken into consideration. Impurities (<0.8%) in the deuterium labeled internal standard and matrix effects may be the reason for an increased LOQ value. As a result, the LOQ for the established protocol was estimated to be 20 pg/µL.

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Table S1. 20 Repeat measurements analyzing standard spiked into blank matrix at the LoD concentration of 5 ppb. Note that the blank signal is at 1.36E+02 and noise (standard deviation) is 38. Even if we consider the blank signal as noise, 19 out of 20 of these duplicate runs yielded a signal higher than 3 times the noise

Exp #	IPA BG	Signal	Sig-Bg	>blank+3Noise	>3Blank
1	2.96E+02	1.45E+03	1.15E+03	TRUE	TRUE
2	2.96E+02	2.43E+03	2.13E+03	TRUE	TRUE
3	2.96E+02	2.90E+03	2.60E+03	TRUE	TRUE
4	2.45E+02	3.11E+03	2.87E+03	TRUE	TRUE
5	2.45E+02	4.61E+03	4.37E+03	TRUE	TRUE
6	2.45E+02	3.93E+03	3.69E+03	TRUE	TRUE
7	2.15E+02	1.40E+03	1.19E+03	TRUE	TRUE
8	2.15E+02	2.65E+03	2.44E+03	TRUE	TRUE
9	2.15E+02	5.65E+03	5.44E+03	TRUE	TRUE
10	1.74E+02	1.15E+03	9.76E+02	TRUE	TRUE
11	1.74E+02	1.50E+03	1.33E+03	TRUE	TRUE
12	1.74E+02	2.44E+03	2.27E+03	TRUE	TRUE
13	1.53E+02	5.21E+02	3.68E+02	TRUE	FALSE
14	1.53E+02	9.39E+02	7.86E+02	TRUE	TRUE
15	1.53E+02	1.65E+03	1.50E+03	TRUE	TRUE
16	4.38E+02	2.63E+03	2.19E+03	TRUE	TRUE
17	4.38E+02	4.54E+03	4.10E+03	TRUE	TRUE
18	4.38E+02	5.98E+03	5.54E+03	TRUE	TRUE
19	9.38E+01	6.28E+02	5.34E+02	TRUE	TRUE
20	9.38E+01	1.88E+03	1.79E+03	TRUE	TRUE

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4. Quantitation using quadrupole ion trap mass spectrometer operated using conventional (consecutive) multiple reaction monitoring (MRM) mode

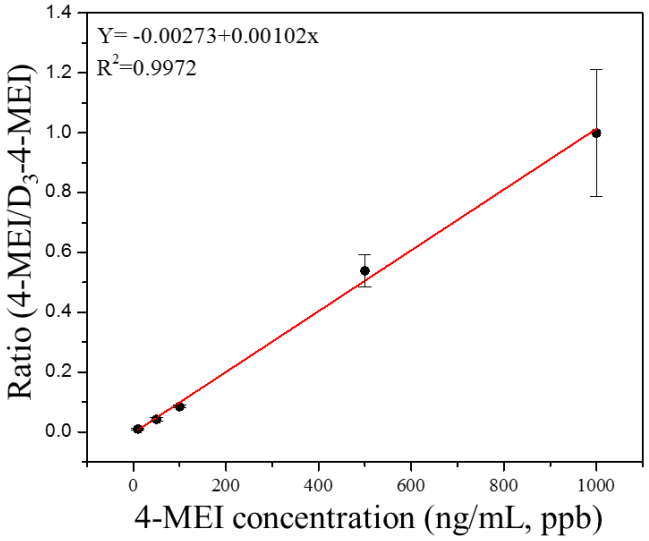

Figure S3. Calibration curves for 4-MEI ionized by paper spray mass spectrometry performed directly on diluted caramel sample and recorded using a linear ion trap. The concentration of internal standard (D_3-4-MEI) was held constant at 1 µg/mL (ppm) and the quantitation range was 10 ~ 1000 ng/mL (ppb).

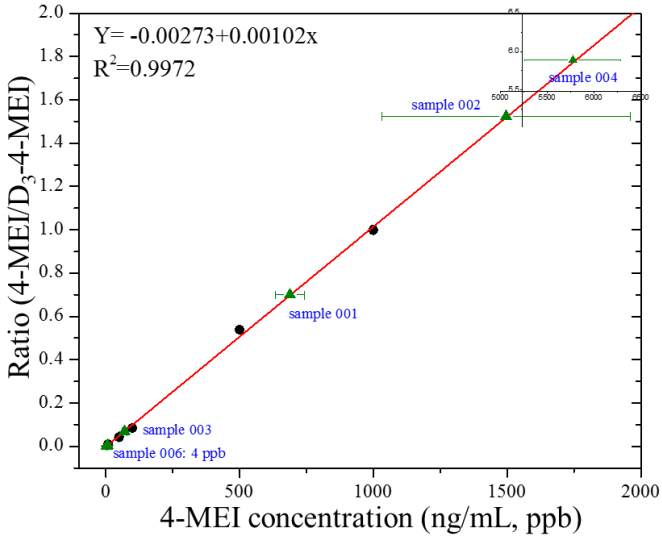

Figure S4. Analysis of 4-MEI in diluted caramel samples using a linear ion trap. The concentration of the internal standard (D_3-4-MEI) was 1 ppm. The circles represent calibration points while the triangles represent measurements on some of the caramel samples. The error bars show standard deviations from three duplicate runs. The point at 1496 and 5775 (in the insert top right) falls outside the range (1-1000 ng/mL (ppb) of calibration.

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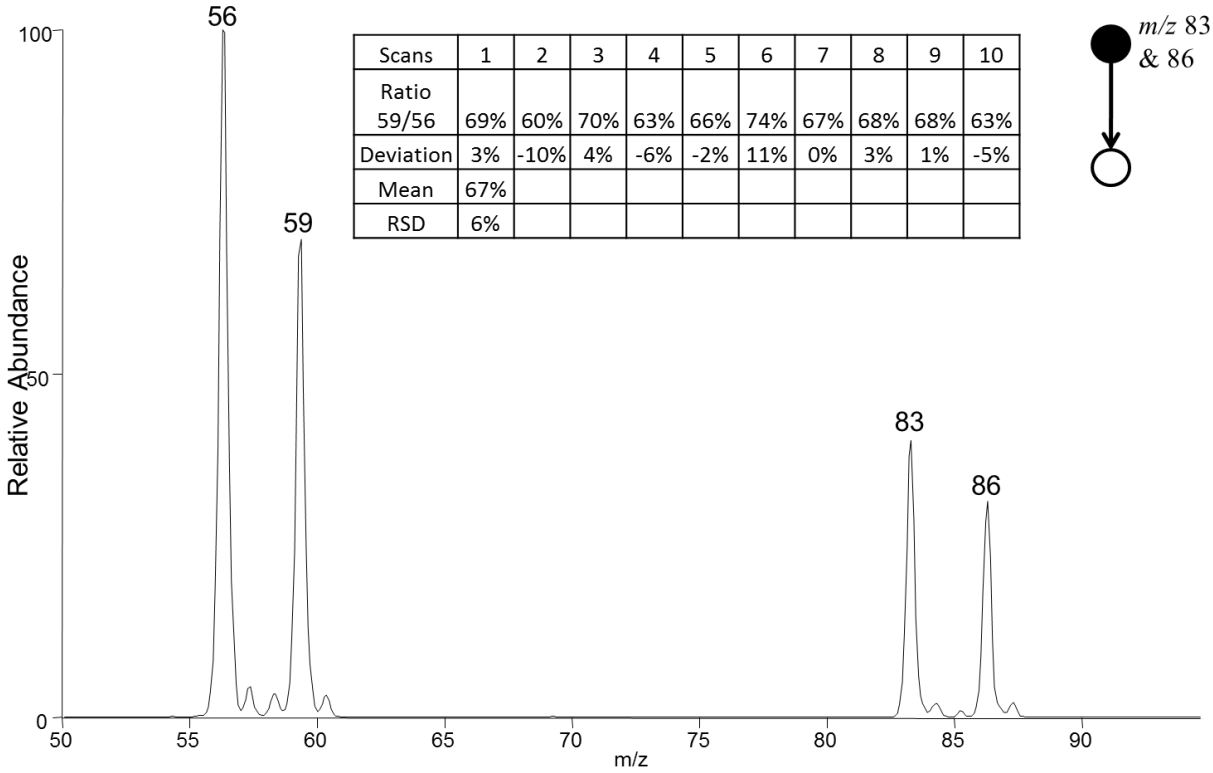
5. Comparing results from nanoESI and paper spray ion sources in the ion trap simultaneous MRM experiment

Simultaneous MRM works by isolating both the analyte and internal standard using a wide isolation window (6 mass units centered at *m/z* 84.5, $q_z = 0.5$). A nominal collision energy of 40% (arbitrary units) was used to fragment all the isolated ions. The product ions were mass analyzed by the following full scan. In this way, the two MRM transitions were monitored in the same scan event, instead of switching between the two SRM channels. A typical simultaneous MRM spectrum is shown with the results of ten consecutive scans as inset, in Figure S5. (A blank matrix run was also carried out to verify that there is no other ionic species would fragment under this simultaneous MRM condition to *m/z* 56 & 59.)



Scans	1	2	3	4	5	6	7	8	9	10
Ratio 59/56	69%	60%	70%	63%	66%	74%	67%	68%	68%	63%
Deviation	3%	-10%	4%	-6%	-2%	11%	0%	3%	1%	-5%
Mean	67%									
RSD	6%									

Figure S5. Ion trap simultaneous MRM for both the analyte (m/z 83→56) and the internal standard (m/z 86→59). The inset table shows that the consistency of the intensity ratios in ten consecutive scans when analyzing a typical calibration standard using paper spray.

Using this method, the analytical precision has been significantly improved. Shown in Table S2, he RSDs of the analysis results from paper spay are very close to that from nanoESI, which is an ionization source with a more stable total ion current.

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Table S2. Using ion trap simultaneous MRM, paper spray analysis results compared with those obtained by nanoESI. %RSD: relative standard deviation in percentage.

Analysis results (µg/mL) returned by different modes using ion trap						
	paperspray, sMRM		nanoESI, ITsMRM		paperspray, MRM	
Sample	Mean	% RSD	Mean	% RSD	Mean	% RSD
1	55.6	1.8%	55.3	0.8%	68.9	8.0%
2	149.2	1.8%	142.1	1.7%	149.6	30.9%
3	7.3	2.3%	6.6	0.1%	7.1	11.8%
4	454.9	2.7%	447.2	0.7%	557.5	8.9%
5	13.7	8.1%	14.7	4.5%	Not available	
7	76.2	9.2%	74.9	2.9%		
8	354.5	2.0%	351.0	2.0%		
9	41.3	2.7%	45.0	1.8%		
10	498.6	0.8%	496.1	1.2%		
11	208.5	1.6%	202.5	0.3%		
12	7.6	10.1%	9.0	10.3%		

6. Comparison of qualitative and quantitative results from different mass spectrometers

The results of paper spray 4-MEI analysis using three types of mass spectrometer are summarized in the Table S3. As is shown, the triple quadrupole mass spectrometer returns values with very small standard deviation, compared to the results from the quadrupole ion trap. Full scan screening using an Orbitrap mass spectrometer provides comparable qualitative data. Its sensitivity decreases when isotopic matching is required, which results in false negative as shown in the Orbitrap Screening column.

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Table S3. Comparison of results obtained using three different mass spectrometers. %RSD: relative standard deviation in percentage.

Analysis results (µg/mL) returned by mass spectrometers						
	Ion trap (LTQ)		triple quad (TSQ)		Orbitrap Screening	
Sample	Mean	% RSD	Mean	% RSD	Screen 2	Screen 1
1	55.6	2%	57.3	0%	Positive	Positive
2	149.2	2%	140.1	5%	Positive	Positive
3	7.3	2%	6.3	13%	Negative	Positive
4	454.9	3%	447.7	1%	Positive	Positive
5	13.7	8%	14	4%	Not available	
7	76.2	9%	70.4	10%		
8	354.5	2%	346.2	2%		
9	41.3	3%	43.9	1%		
10	498.6	1%	490.2	1%		
11	208.5	2%	193.1	2%		
12	7.6	10%	6.5	7%		

[a]Req 2: analyte and its ^{13}C peak observed; Screen 1: analyte peak observed

7. Possible use of flow injection analysis as an alternative to paper spray

A referee has asked whether flow-injection ESI (FIA-ESI) might be considered as an alternative to PS. We have not tried to use syringe-pumped direct infusion ESI because food samples are too complex and concentrated, and likely will contaminate the syringe and result in memory effects capillary loop and the ESI source. We did experiments using a disposable direct infusion ESI method— nanoESI using a pulled glass capillary (Table S2). This experiment has several drawbacks that significantly reduced throughput. 1) Loading the aqueous samples to the tapered tip is difficult because of the large surface tension of water compared to solvents. 2) Even though one can dispose of the nanoESI tip after each measurement, one still needs to clean the electrode which is in contact with the sample solution. And after cleaning, one must run a blank using another nanoESI tip. Compared to the paper spray protocol, this takes much more time. 3) To successfully perform ESI of the aqueous sample (high surface tension), higher voltage or smaller spray tip head is needed. These facts make clogging problem more severe especially considering high salt/sugar content samples such as soy sauce and cola drinks. 4) Cola drinks are usually carbonated, so if sampled in-situ, CO_2 bubbling will occur inside the spray tip, and this is detrimental to the quality of the spray signal. 5) Some of the caramel samples are so viscous that it is impossible to load them into a nanoESI or any other direct infusion ESI source. None of these problems are a concern when using porous disposable paper triangles. So, even though slightly better precision was achieved with nanoESI, as shown in Table S2, it appears not be widely applicable for high throughput analysis of these types of sample.