Standard addition strip for quantitative electrostatic spray ionization mass spectrometry analysis: Determination of caffeine in drinks.

-Supporting Information-

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SI-1: Limit of detection of caffeine by ESTASI-MS.

5 μl of caffeine (51 nM) in ESI buffer (50% methanol, 49% water and 1% acetic acid) was deposited on the plastic plate for ESTASI-MS analysis. The mass spectrum was recorded in a positive ion mode.

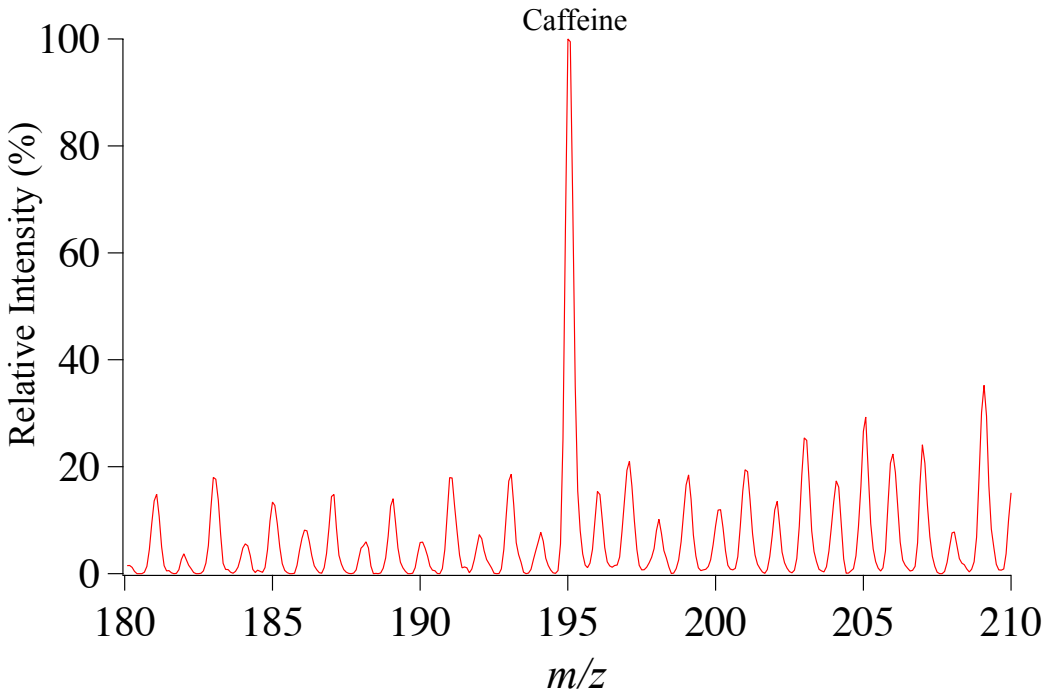


Figure SI-1. Mass spectrum of caffeine with the concentration of 51 nM by ESTASI-MS.

SI-2: Quantitative analysis from droplets of standard solution by ESTASI-MS

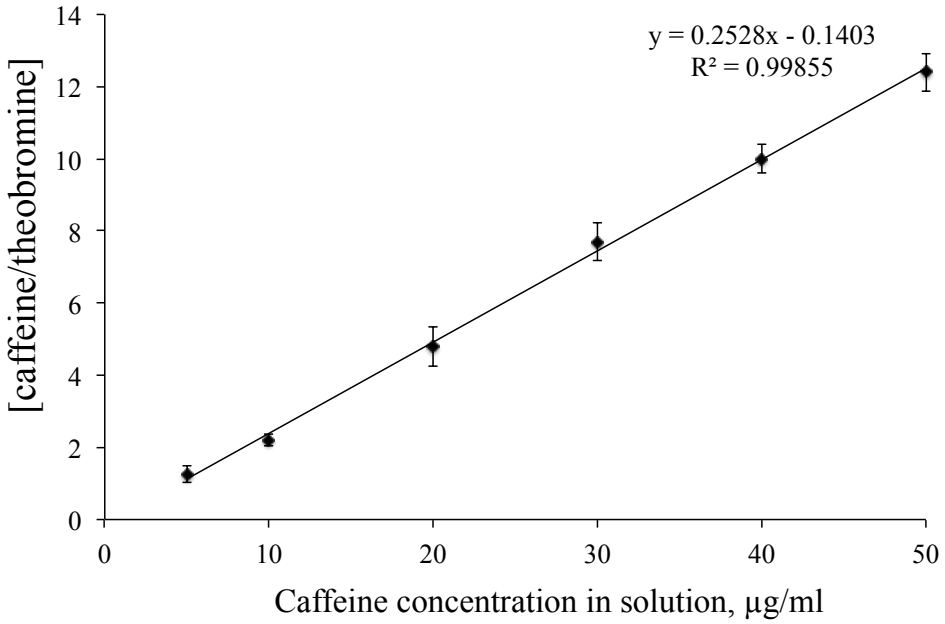


Figure SI-2.1. The mass spectral peak intensity ratio between caffeine and theobromine as a function of the caffeine concentration obtained by ESTASI-MS. Error bar shows the standard deviation calculated from three experiments.

SI-3: Standard addition calibration of caffeine in various drinks by ESTASI-MS or liquid chromatography.

Solutions containing 5-50 μg/mL of caffeine, constant amount of theobromine (final concentration: 5 μg/ml) and 50 times diluted beverages (Coffee/tea infusions, Coca-Cola Classic, Coca-Cola Zero, Ice Tea) in the acidic solution (50% methanol, 49% water and 1% acetic acid) were deposited on the plastic plate for ESTASI-MS analysis. All mass spectra were obtained in a positive ion mode and the ratios between caffeine and theobromine peak intensities were used to plot against concentration of added pure caffeine.

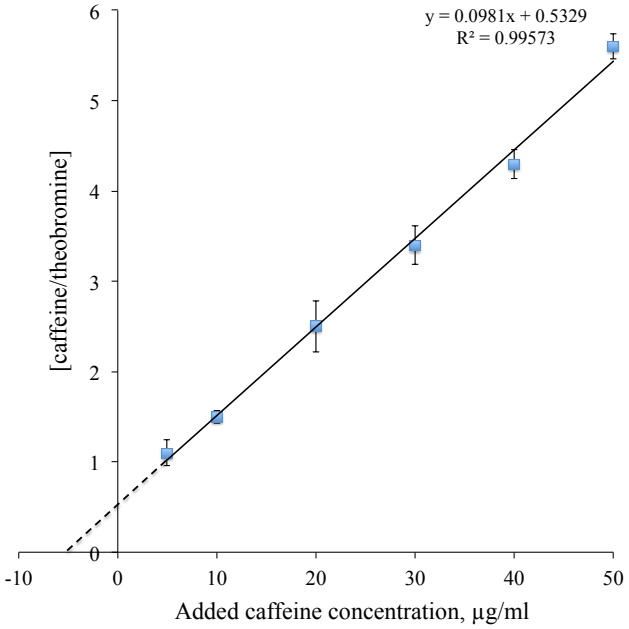


Figure 3.1. Black coffee sample, plot of caffeine/theobromine single protonated peak intensity ratio as a function of the added caffeine concentrations obtained by ESTASI-MS. Error bar shows the standard deviation calculated from three experiments

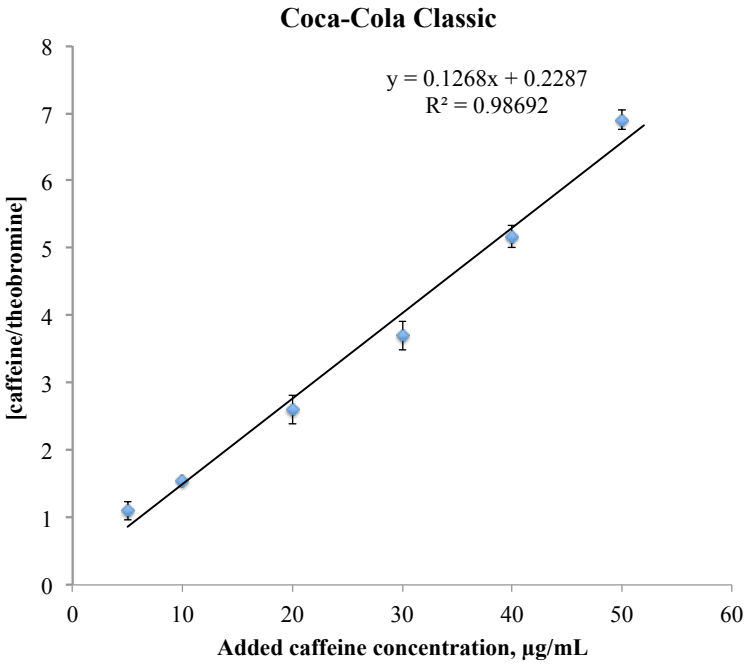


Figure SI-3.2. Coca-Cola Classic sample, the plot of caffeine/theobromine signal intensity ratio as a function of the added caffeine concentrations obtained by ESTASI-MS. Error bar shows the standard deviation calculated from three experiments.

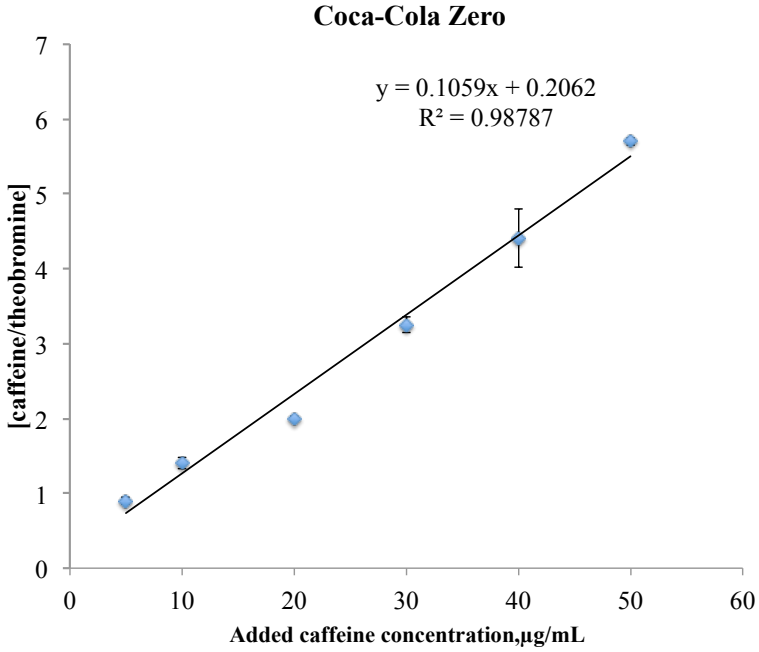


Figure SI-3.3. Coca-Cola Zero sample, the plot of caffeine/theobromine signal intensity ratio as a function of the added caffeine concentrations obtained by ESTASI-MS. Error bar shows the standard deviation calculated from three experiments.

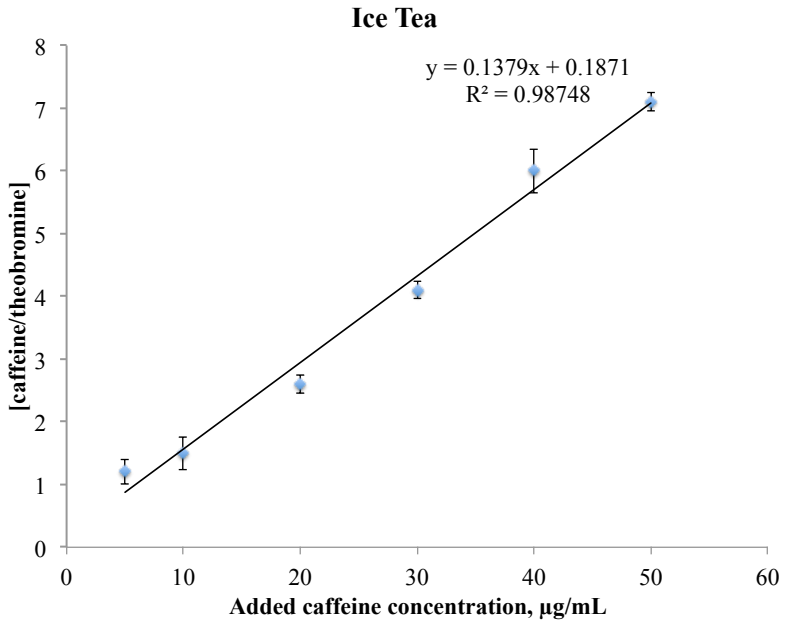


Figure SI-3.4. Ice tea ("Nestea" lemon), the plot of caffeine/theobromine signal intensity ratio as a function of the added caffeine concentrations obtained by ESTASI-MS. Error bar shows the standard deviation calculated from three experiments.

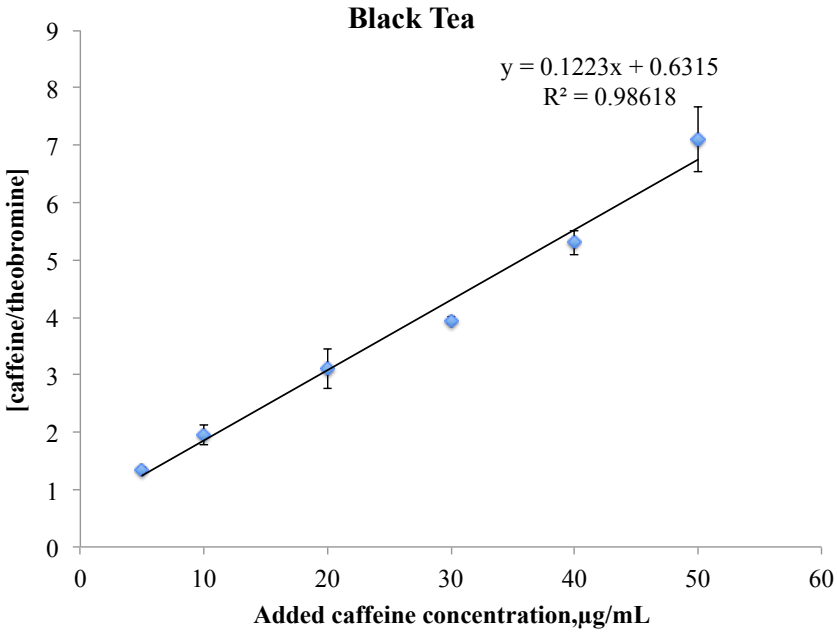


Figure SI-3.5. Black tea extract ("Lipton") sample, the plot of caffeine/theobromine signal intensity ratio as a function of the added caffeine concentrations obtained by ESTASI-MS. Error bar shows the standard deviation calculated from three experiments.

Seven samples for black tea as well as for black coffee were prepared with different amounts of added pure caffeine. Soluble coffee "Nescafe Gold" (2 g) was diluted in 100 ml of boiled water. Black tea leaves "Lipton" (2 g) were poured for 5 minutes with 100 ml of boiling water and filtered. The sample of black coffee and tea were diluted 50 times, and pure caffeine was added to the samples to form a final concentration from 5 to 50 µg/ml. 20 µl of each solution was injected to HPLC, and separated with a mobile phase of methanol/water 50/50 (v/v) under a flow rate of 0.8 ml/min. The experiments for each sample were repeated 3 times.

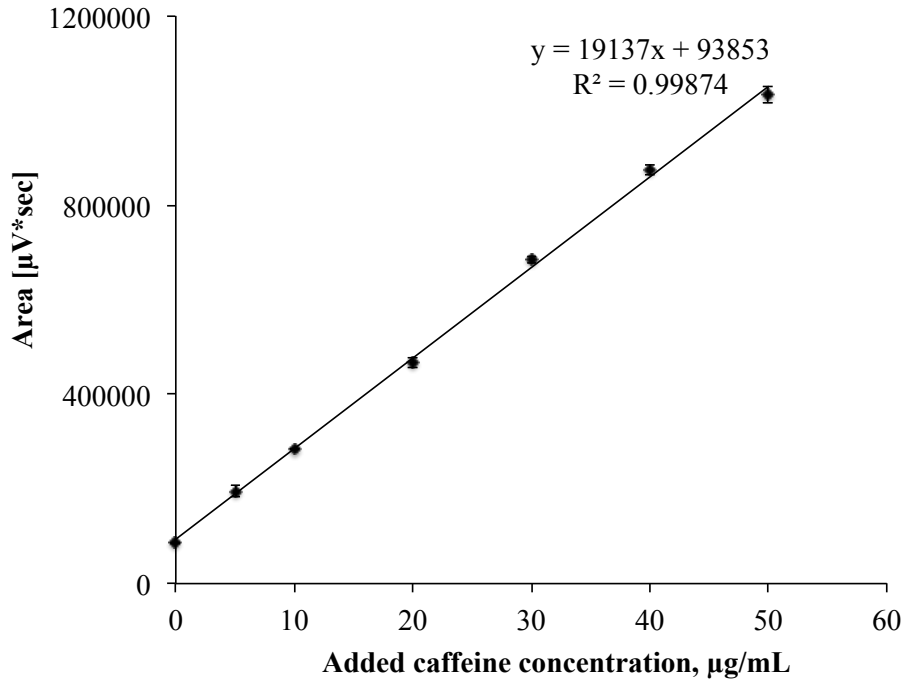


Figure SI-3.6. Black tea extract ("Lipton") sample, the plot of average area of a caffeine peak as a function of the added caffeine concentrations obtained by HPLC. Error bar shows the standard deviation calculated from three experiments.

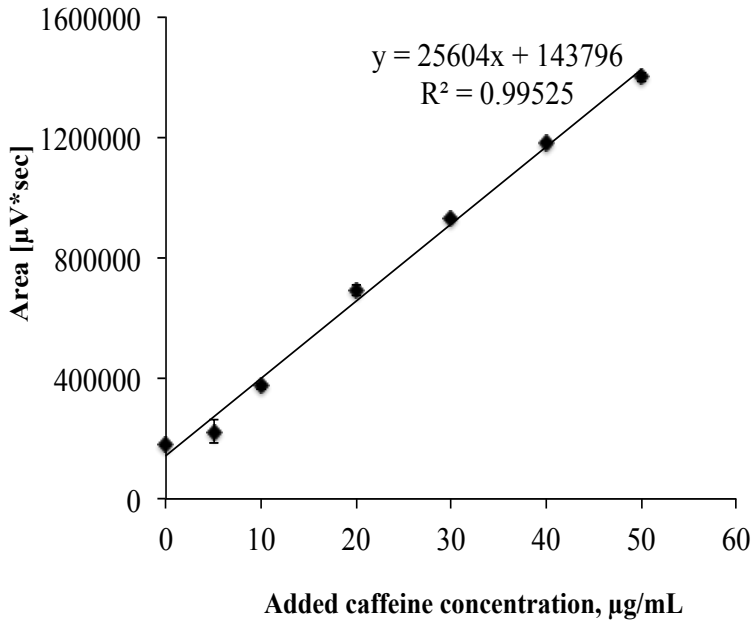
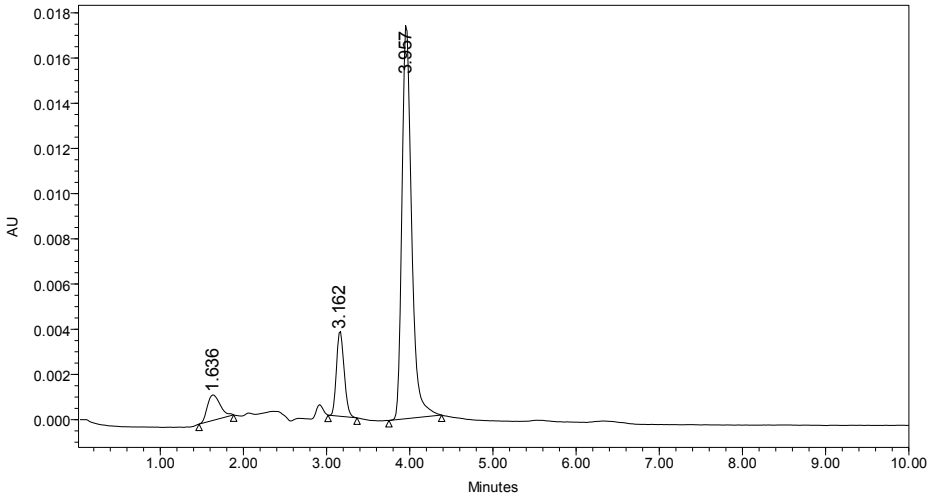


Figure SI-3.7. Coffee ("Nescafe Gold") sample, the plot of average area of a caffeine peak as a function of the added caffeine concentrations obtained by HPLC. Error bar shows the standard deviation calculated from three experiments.

SAMPLE INFORMATION

Sample Name:	solution1Unk.	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	29.04.2014 13:45:19
Vial:	1	Acq. Method:	IPS
Injection #:	1	Processed By:	System
Injection Volume:	5.00 ul	Date Processed:	29.04.2014 13:56:20
Run Time:	10.00 Minutes	Channel Name:	2487Channel 2
Sampling Rate:	1.00 per sec	Channel Desc.:	
		Sample Set Name:	

Sample Values
Used in Calculations:



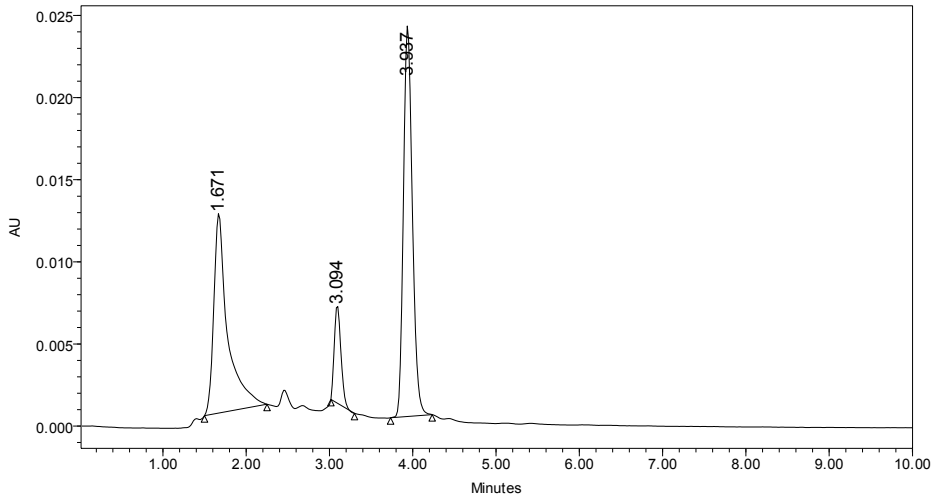
	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)
1	1.636	Unknown	12022	6.79	1129	5.04	bb	25	1.467	1.883	1.467
2	3.162	Unknown	24168	13.65	3759	16.80	bb	21	3.017	3.367	3.017
3	3.957	Unknown	140819	79.55	17493	78.16	bb	37	3.750	4.383	3.750

Figure SI-3.8.1 Chromatogram obtained for the determination of caffeine in a black tea sample using 50/50 MeOH/water as the mobile phase. The peak at 3.957 min corresponds to caffeine.

SAMPLE INFORMATION

Sample Name:	coffee 1.1 Unk.	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	30.04.2014 10:16:01
Vial:	1	Acq. Method:	IPS
Injection #:	9	Processed By:	System
Injection Volume:	5.00 ul	Date Processed:	30.04.2014 10:36:59
Run Time:	10.00 Minutes	Channel Name:	2487Channel 2
Sampling Rate:	1.00 per sec	Channel Desc.:	
		Sample Set Name:	

Sample Values
Used in Calculations:



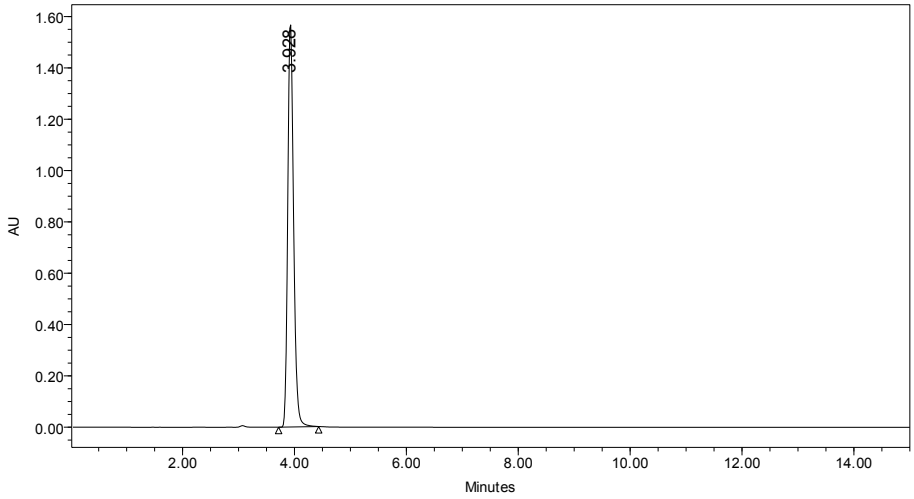
	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Points Across Peak	Start Time (min)	End Time (min)	Baseline Start (min)
1	1.671	Unknown	140478	40.00	12132	29.02	bb	45	1.500	2.250	1.500
2	3.094	Unknown	32952	9.38	5902	14.12	bb	16	3.017	3.300	3.017
3	3.937	Unknown	177730	50.61	23767	56.86	bb	30	3.733	4.233	3.733

Figure SI-3.8.2. Chromatogram obtained for the determination of caffeine in a black coffee sample using 50/50 MeOH/water as the mobile phase. The peak at 3.937 min corresponds to caffeine.

SAMPLE INFORMATION

Sample Name:	caffeine 5050Unk.	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	29.04.2014 10:37:33
Vial:	1	Acq. Method:	IPS
Injection #:	1	Processed By:	System
Injection Volume:	5.00 ul	Date Processed:	29.04.2014 13:59:09
Run Time:	15.00 Minutes	Channel Name:	2487Channel 2
Sampling Rate:	1.00 per sec	Channel Desc.:	
		Sample Set Name:	

Sample Values
Used in Calculations:



	RT (min)	Peak Type	Area (µV*sec)	% Area	Height (µV)	% Height	Integration Type	Points Across Peak	Start Time (min)	End Time (min)
1	3.928	Unknown	11406046	100.00	1571351	100.00	bb	43	3.717	4.433

Figure SI-2.8.3 HPLC chromatogram of 0.5 mg/ml pure caffeine using 50/50 MeOH/water as the mobile phase.

SI-4: Method of standard addition using strip-ESTASI-MS

20 μl of a beverage (Coca-Cola Classic, Coca-Cola Zero, Ice Tea or black coffee/tea infusion) were mixed with 25 μl of theobromine (initial concentration 200 μg/ml). Afterwards, the mixture was diluted to 1 ml by the acidic solution (50% methanol, 49% water and 1% acetic acid). The solution (5 μl) was deposited on the wells of a plastic strip, containing the spots of caffeine dried from 5 μl of solution with the concentrations in a range of 5 to 50 μg/ml. The peak intensity ratios between caffeine/theobromine were obtained by ESTASI-MS to plot the calibration curves.

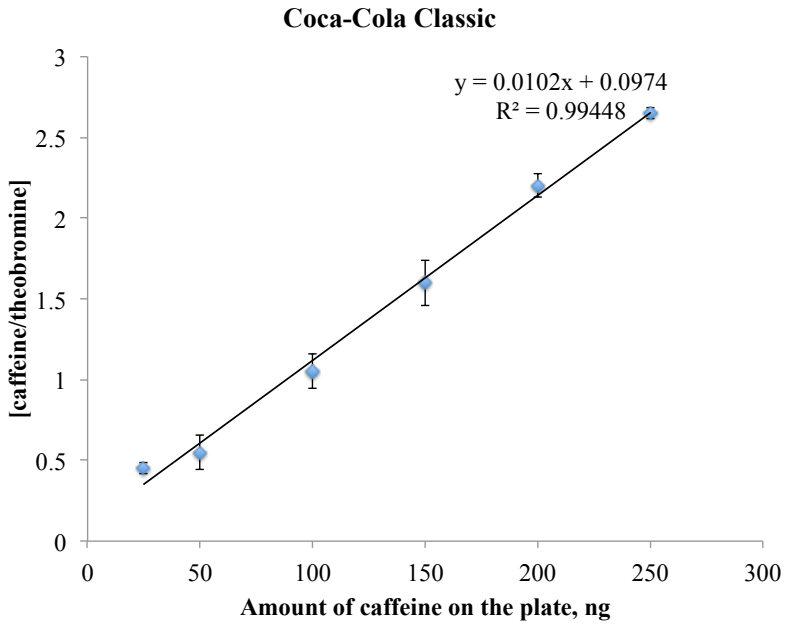


Figure SI-4.1. Coca-Cola Classic sample, the plotting of caffeine/theobromine signal intensity ratio as a function of the amount of caffeine in the dried spot by the strip-ESTASI-MS standard addition method. The standard deviation was calculated from thee experiments and shown as the error bar.

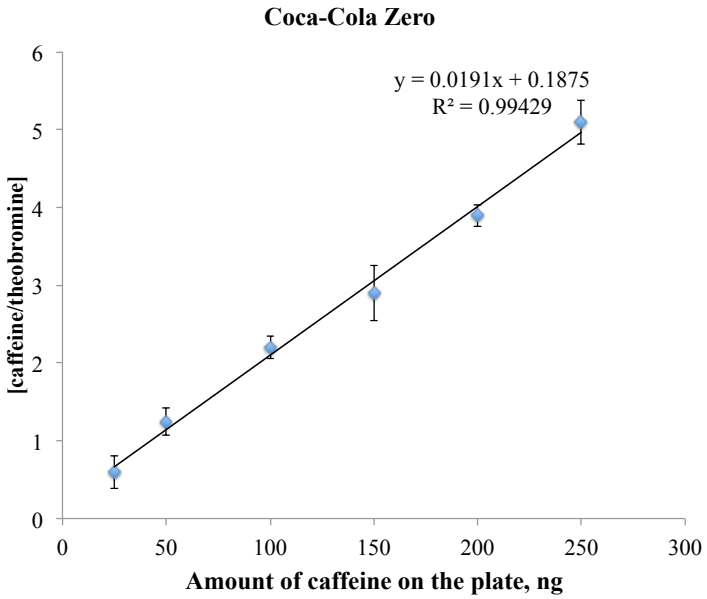


Figure SI-4.2. Coca-Cola Zero sample, the plotting of caffeine/theobromine signal intensity ratio as a function of the amount of caffeine in the dried spot by the strip-ESTASI-MS standard addition method. The standard deviation was calculated from thee experiments and shown as the error bar.

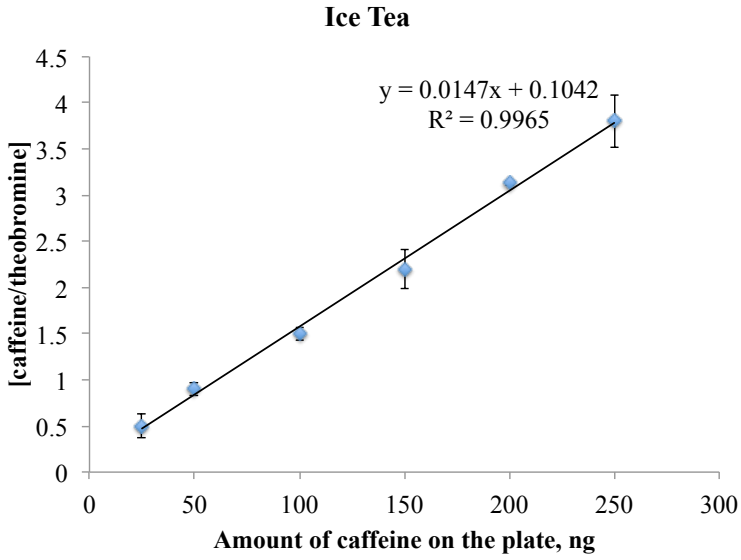


Figure SI-4.3. Ice Tea ("Nestea" lemon) sample, the plotting of caffeine/theobromine signal intensity ratio as a function of the amount of caffeine in the dried spot by the strip-ESTASI-MS standard addition method. The standard deviation was calculated from thee experiments and shown as the error bar.

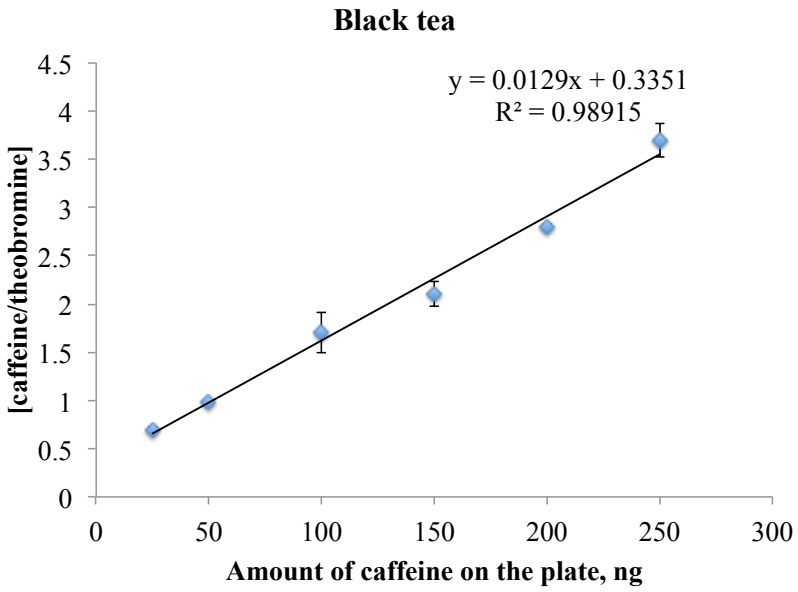


Figure SI-4.4. Black tea infusion ("Lipton") sample, the plotting of caffeine/theobromine signal intensity ratio as a function of the amount of caffeine in the dried spot by the strip-ESTASI-MS standard addition method. The standard deviation was calculated from thee experiments and shown as the error bar.

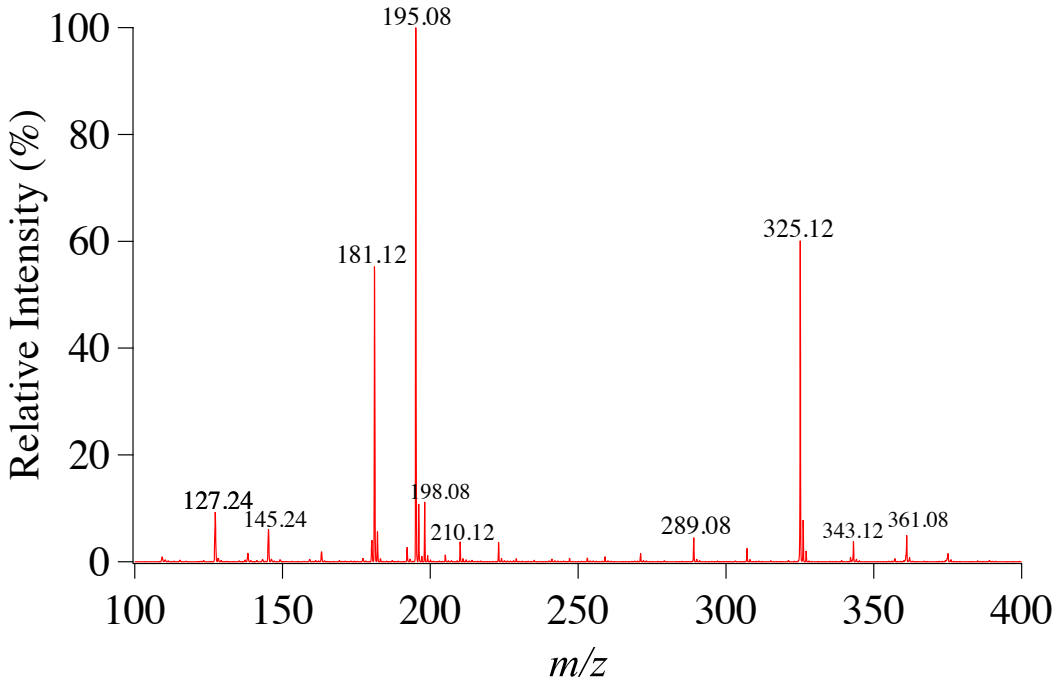


Figure SI-4.5. Mass spectra of Nestea Lemon obtained by strip-ESTASI-MS. The caffeine amount on the strip was 50 ng. The MS data was obtained in a positive ion mode.

To verify that the previously dried pure caffeine can be fully extracted from the wells of a strip during ESTASI-MS analysis, series of experiments were performed. Droplets (5 μl each) of caffeine/theobromine mixture in the acetic solution containing different concentrations of pure caffeine (5, 10, 20, 30, 40, 50 μg/ml) and a fixed concentration of theobromine (5 μg/ml) were deposited on a blank strip and a strip with previously dried caffeine spots. The mass spectral intensity ratios between caffeine and theobromine were calculated and plotted as a function of the caffeine concentration as shown on Figure 4.6. Curve A with a gradient of 0.2528 was obtained from the blank strip; while curve B with a gradient of 0.5003, almost double of curve A, was obtained from the strip with previously dried spots containing 25, 50, 100, 150, 200, 250 ng of caffeine, respectively. Such a result indicates that almost all previously dried caffeine on the spots was quickly dissolved into the acidic droplet for ESTASI-MS analyses.

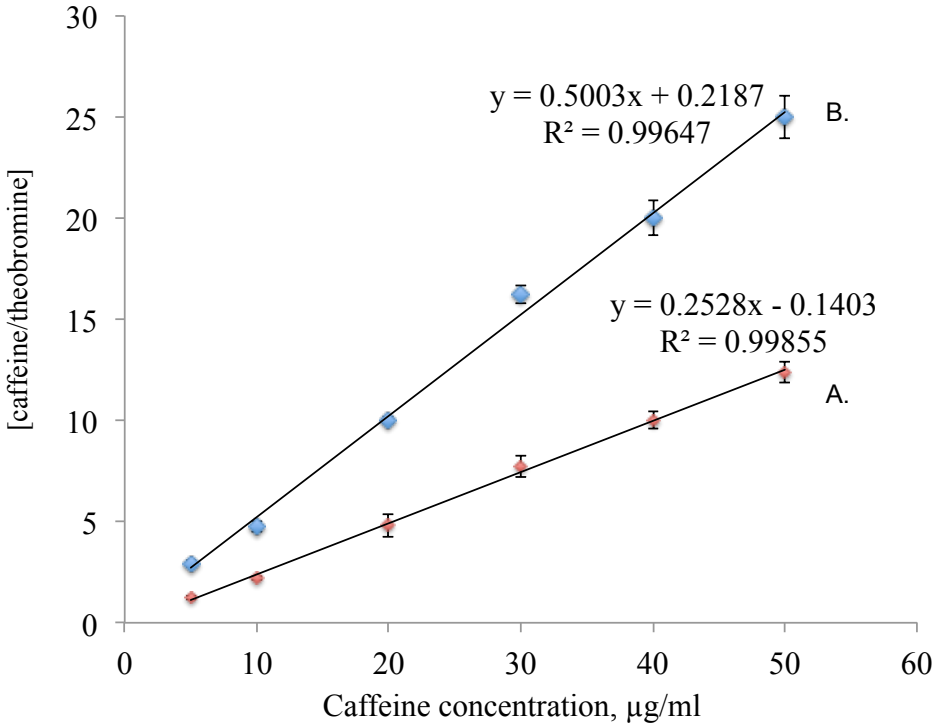


Figure SI-4.6. The mass spectral peak intensity ratio between caffeine and theobromine as a function of the caffeine concentration in the droplets on a blank strip (A) and on a strip containing previously dried caffeine spots (B). Error bar shows the standard deviation calculated from three experiments.

SI-5: Quantification of caffeine in saliva by strip-ESTASI MS

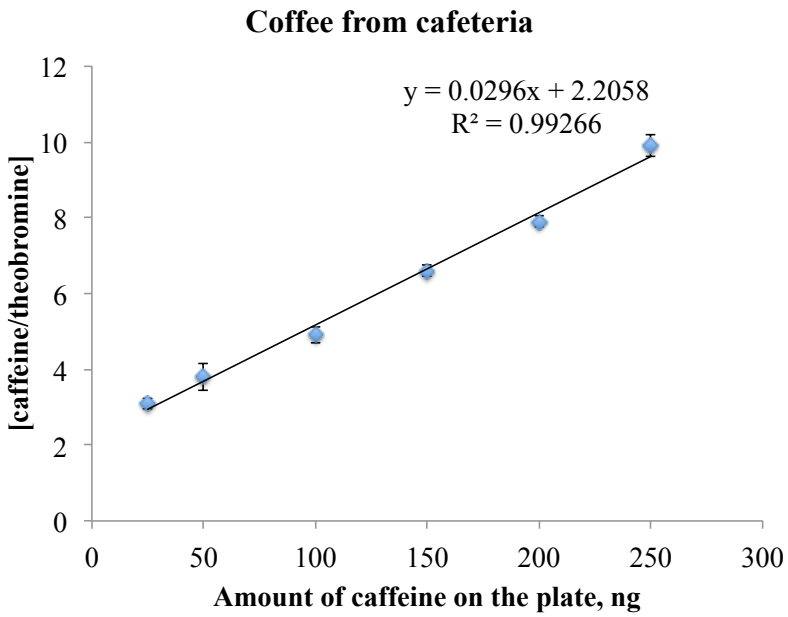


Figure SI-5.1. The plotting of caffeine/theobromine signal intensity ratio as a function of the amount of caffeine in the dried spot by the strip-ESTASI-MS standard addition method. The standard deviation was calculated from thee experiments and shown as the error bar. The saliva sample was collected directly after drinking a cup of coffee from the local cafeteria