

Quantitative TLC/MS of Caffeine Using Surface Sampling Electrospray Ionization Mass Spectrometry

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OVERVIEW

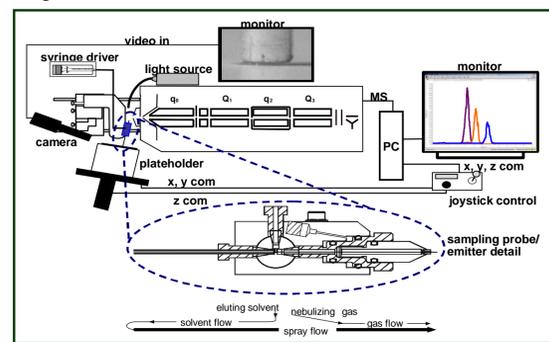
- The quantitative determination of caffeine on reversed-phase (RP) C8 TLC plates using a surface sampling electrospray ionization system with tandem mass spectrometry is reported.
- Quantitation was performed using thin layer chromatography /electro-spray tandem mass spectrometry. The method featured deuterium-labeled caffeine internal standard and selected reaction monitoring detection.
- Limits of detection for the proposed method and a reference HPLC/UV procedure developed in parallel were 1.0 ng spotted on the plate and 0.2 ng injected (0.50 μ L), respectively.
- Spike recoveries with standards and real samples ranged between 97-106% for both methods.
- The HPLC/UV and TLC/MS determinations were in general agreement for three diet soft drinks and three sport drinks tested.

INTRODUCTION

- Thin layer chromatography (TLC) can be an adaptable and inexpensive approach to analytical separation challenges.
- TLC is versatile, affordable, and used routinely in the chemical and life sciences.
- Coupling TLC with mass spectrometry (MS) pairs a very simple and robust separation method with a detector capable of selectively detecting a very wide variety of species at trace- to ultratrace levels.
- Highly-specific quantitative TLC is one additional advantage made possible with the coupling of TLC to MS detection.
- Reported quantitative TLC/MS methods lack analysis of real samples and/or comparison of resulting quantitative values with those generated using reference methods.
- Our work demonstrates the determination of caffeine using a surface sampling probe electrospray (ES) ionization system to couple TLC with tandem mass spectrometric detection.
 - Caffeine is a small polar molecule, suitable for both positive-ion electrospray ionization and separation on reversed-phase TLC.
 - Caffeine is an ingredient in a wide variety of commercially-available beverages.
 - A low-cost stable isotope internal standard is available.
 - An HPLC method with UV detection was developed in parallel as a check on the TLC/ES-MS/MS results.

EXPERIMENTS

- Samples**
 - The caffeine content of three cola drinks and three sport drinks were analyzed for their caffeine content (see data table).
 - Portions of the samples were diluted, filtered through a 0.45 μ m porosity syringe filter, then analyzed immediately using the HPLC/UV method.
- Thin-Layer Chromatography (TLC)**
 - TLC was performed using 10 x 10 cm glass-backed RP C8 plates, which were developed using 70/30 (v/v) methanol/water
 - Samples and standards were spotted manually with a micro pipet (1.0 μ L) in parallel development lanes. Samples intended for mass spectrometric analysis were fortified with 50 ng caffeine- d_3 (1-methyl- d_3) internal standard.
- Surface Sampling System**
 - A surface sampling electrospray probe was mounted on a 4000 Q Trap hybrid triple quadrupole linear ion trap mass spectrometer, as shown in Figure 1, below.



- A robotic x,y,z sample handling platform, modified to accommodate a 10 x 10 cm TLC plate, held and positioned the plate in the x,y plane perpendicular to the z-plane of the sampling probe.
- A syringe pump delivered 60/40 (v/v) methanol/water (0.1% (v/v) acetic acid) eluting solvent to the surface sampling probe.
- The surface was scanned under computer control horizontally relative to the stationary probe across the caffeine bands in parallel development lanes at a constant scan rate of 40 μ m/second. The z-position of the surface was operated manually to maintain a 20-50 μ m thickness liquid junction for optimum surface sampling.
- Mass Spectrometric Analysis**
 - The mass spectrometer was operated in the selected reaction monitoring (SRM) mode.
 - The SRM transitions monitored were m/z 195 \rightarrow 138 and m/z 195 \rightarrow 110 (for caffeine) and m/z 198 \rightarrow 138 and m/z 198 \rightarrow 110 (for caffeine- d_3).
 - The dwell time for each transition was 250 ms.
- HPLC/UV Reference Procedure for Caffeine**
 - All HPLC determinations were performed using an Agilent 1100 capillary LC system
 - The eluent (50/50/0.5 v/v/v methanol/water/acetic acid) was filtered through a 0.45 μ m porosity nylon filter.
 - Samples (0.5 μ L injection) were eluted at 25 μ L/min from a 250 x 1 mm column packed with Partisil ODS-3 (C18, 5 μ m particle diameter).
 - Quantitation was based on the absorbance measured at 275 nm.

RESULTS AND DISCUSSIONS

Sample	Literature ¹	HPLC/UV Reference Method					TLC/ES-MS/MS Method				
		Mean	Mean vs. lit. %	Standard Deviation	% RSD	Replicates	Mean	Mean vs. lit. %	Standard Deviation	% RSD	Replicates
Diet Coke	45	46.0	+2.2	0.3	0.7	4	43.2	-4.0	0.7	1.6	3
Diet Pepsi	36	35.0	-2.8	0.2	0.7	4	34.7	-3.6	0.8	2.3	3
Diet Cherry Coke	34	36.9	+8.5	0.3	0.9	4	38.0	+12	0.8	2.1	3
Diet Turbo Tea®	90	120.8	+34	0.7	0.6	4	119.8	+33	0.8	0.7	3
Speed Stack™ Grape	250	276.2	+10	0.7	0.2	4	270.0	+8.0	11	1.5	3
Speed Stack™ Fruit Punch	250	284.4	+14	0.7	0.25	4	278.0	+11	4.3	4.4	3

¹Literature values for Diet Coke, Diet Pepsi, and Diet Cherry Coke taken from "American Beverage Association, Nutrition & Health, Ingredients, Caffeine". Internet address is <http://www.nsdas.org/health/caffeinecontent.asp>. Last accessed January 13, 2005. Neither the error associated with the label and literature values for caffeine content, nor the details of the methods used to determine those values, is known.

HPLC Results

- The HPLC calibration data (1.0-50 ng/ μ L) were evaluated using a least squares regression, and fit the model $y = (111.31 \pm 0.18)x + (-5.2 \pm 2.1)$, where y is the integrated peak area and x is the mass (ng) of caffeine injected ($r^2 > 0.9999$).
- A typical chromatogram from a 50 ng caffeine/ μ L standard is shown in Figure 2 (t_R for caffeine ~ 9.3 min). The UV spectrum superimposed, taken from the analysis of Diet Turbo Tea®, shows $\lambda_{max} = 275$ nm, consistent with literature data.
- The detection limit was estimated to be 0.20 ng caffeine injected based on $3s_{xy}$ /slope of the calibration line. The parameter $3s_{xy}$ is the standard error of the y-value estimates, and is assumed to approximate the standard deviation of the blank.
- For each sample type, a spike recovery was performed by adding a caffeine spike (final concentration 10 ng/ μ L) as part of the final dilution. Spike recoveries of caffeine ranged between 97-105%, and were consistent with the simple sample preparation method employed. There was no significant contribution from the sample matrix or the analytical procedure itself to the quantitative determination of caffeine.
- An instrumental evaluation of "peak purity" clearly demonstrated that there were no additional components that had co-eluted with caffeine that would contribute any bias to the results given above.

TLC/ES-MS/MS Results

- The addition of the internal standard provided a means to compensate for minor variations in sample preparation and instrument parameters, thereby improving the accuracy of the quantitation.
- The presence of the internal standard also allowed for reliable quantitation when the analyte bands on the TLC plate were not sampled from within the same regions of the bands among the bands in the separate development lanes (i.e., all bands not sampled "dead center"). The R_f values of caffeine and d_3 -caffeine are identical; hence, the behavior of the internal standard models that of the analyte exactly.
- Figure 3 shows SRM chromatograms used for quantitation of standards and samples on two TLC plates. The data collection for Figure 3a, involving nine independent development lanes, was completed in 35 minutes.
- The TLC/ES-MS/MS calibration data (1.0-50 ng per spot) were evaluated using a least squares regression, and fit the model $y = (1.094 \pm 0.005)x + (-0.0024 \pm 0.0023)$, where y is the ratio of the integrated analyte peak area to the integrated internal standard peak area, and x is the caffeine standard amount to internal standard amount ratio ($r^2 > 0.9998$).
- The detection limit, calculated in the same manner as for the HPLC/UV method, was 1.0 ng spotted on the plate. However, the probe sampled not more than 16% of the area near the circular bands. The detection level posed no problem for the determination of caffeine.

Comparison of Sample Data between the HPLC/UV and TLC/ES-MS/MS Methods

- The mean caffeine values determined for Diet Coke and Diet Pepsi using TLC/ES-MS/MS were consistent with the HPLC/UV values and close to the expected values (within 4%).
- The mean caffeine values determined for Diet Cherry Coke and each of the sports drinks using TLC/ES-MS/MS were significantly higher than the expected values. However, for these samples, the HPLC/UV and TLC/ES-MS/MS values were more comparable to each other than to the expected values. The latter condition, employing two completely independent quantitation methods, suggests that the values so produced are closer to "true" values than to the literature values.
- A spike recovery study performed by adding a caffeine spike to an aliquot of Diet Cherry Coke prior to filtration yielded a recovery of 106%. Such a result confirmed the absence of any significant contribution from the sample matrix or the analytical procedure itself to the quantitative determination of caffeine.

DATA FIGURES

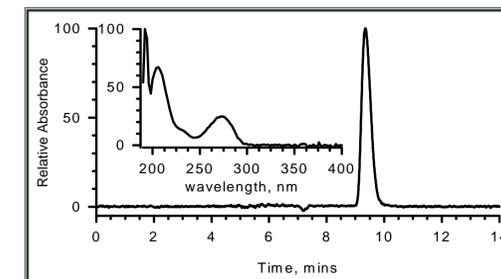


Figure 2. A HPLC/UV chromatogram from a 5 ng injection of a caffeine standard, recorded at 275 nm. Conditions described under "Experiments". The UV spectrum of caffeine (inset) was taken from the chromatographic peak at 9.3 minutes.

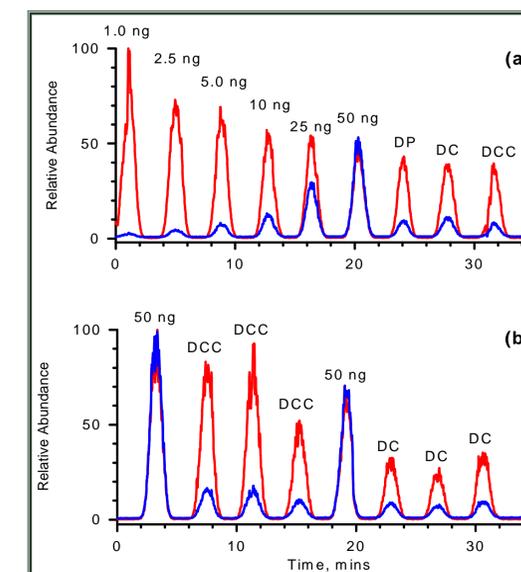


Figure 3 (top), TLC/ES-MS/MS mass chromatograms from the analysis of; (from left to right) 1, 2.5, 5, 10, 25 and 50 ng caffeine standards and Diet Pepsi, Diet Coke and Diet Cherry Coke samples. (bottom), TLC/ES-MS/MS mass chromatograms from the analysis of; (from left to right) a 50 ng/ μ L caffeine standard, three replicate Diet Cherry Coke sample spots, a 50 ng/ μ L caffeine standard and three replicate Diet Coke sample spots. All samples and standards contained 50 ng/ μ L caffeine- d_3 as an internal standard. The blue trace represents the m/z 195 \rightarrow m/z 138 SRM transition monitored for caffeine. The red trace represents the m/z 198 \rightarrow m/z 138 SRM transition monitored for caffeine- d_3 .

CONCLUSIONS

- The quantification of caffeine at the low ng level can be performed directly from the surface of a TLC plate using a surface sampling probe, ES mass spectrometry employing SRM detection, and an isotopically-labeled internal standard spotted with the samples.
- The analyte concentrations so calculated exhibit accuracy comparable to an HPLC/UV method developed in parallel.
- The limit of detection (1.0 ng spotted) and fifty-fold dynamic range compare favorably with the best quantitative TLC/MS methods published to date using other sampling and ionizing techniques.
- Caffeine was quantified successfully in six commercially-available beverages using only minimal sample preparation.
- The present method has been applied successfully to real samples without post separation processing of the plates.

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