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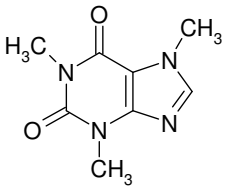
Direct analysis of caffeine in soft drinks and coffee and tea infusions

Caffeine (Figure 1), a xanthine alkaloid acting as psychoactive stimulant and mild diuretic in human, is an integral part of diet of many people. It is often found in natural products such as tea, coffee and cocoa beans, cola nuts and many others. Analysis of caffeine in various foods and beverages is an important task for analytical laboratories, as its content is considered in assessment of product quality (coffee, cocoa beans and tea). Due to its physiological effect, the amount of caffeine is regulated in selected foods in EU. Maximum limits are set for some soft drinks to which caffeine is added. HPLC methods employing UV detection are commonly used for its control. While for soft drinks and coffee/tea infusions, the sample preparation is not too much time demanding, LC separation of sample components becomes a limiting step in laboratory throughput. Employing AccuTOF-DART system offers straightforward examination of caffeine content in tens of samples per hour, thanks to omitting separation step. Isotope dilution is used for target analyte quantification.

Figure 1 Structure of caffeine (1,3,7-trimethylxanthine, CAS Number: 58-08-2).



Experimental

Samples

The samples were prepared for analysis in following way:

(i) Soft drinks (ice tea, cola drink, energy drink) were decarbonized by sonication.
(ii) Soluble coffee 2 g were diluted in 100 mL of boiling water.
(iii) Ground coffee beans and tea leaves (5 g) were extracted with 100 mL of boiling water under shaking (1 min).

All liquid samples were diluted 50 times and 5 µl of aqueous solution containing 5 µg of isotope labeled internal standard ($^{13}C_3$-caffeine) were added to 1 mL of each diluted sample.

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DART-TOFMS measurements

The DART ion source was operated in positive ion mode with helium as the ionizing medium at a flow rate of 2.7 L/min. The gas beam was heated to 300°C, discharge needle voltage set to 3000 V, perforated and grid electrode voltages were +150 V and +250 V, respectively. Accurate mass spectra were acquired in a range of *m/z* 50–500 employing 0.2 s recording interval; the peaks voltage value was set to 1000 V. A solution containing a mixture of poly(ethylene glycol) PEG 600 and 200 was introduced at the end of each sample analysis to compensate any mass drift.

The examined samples were introduced automatically with the use of an AutoDart sampler and Dip-it™ tips. Following steps were involved: (i) sampling tip immersed into the sample; (ii) placing of tip in front of the DART gun exit close to the ion source – mass spectrometer axis; (iii) sampling tip disposed. Five replicate measurements were carried out on examined samples.

Results

As shown in Figure 3, both caffeine and isotope labeled internal standard were detected as $[M+H]^+$ ions. The differences between exact and measured masses were as low as -0.7 mmu and -0.6 mmu, respectively.

In Figure 4 calibration plot of caffeine is shown. Each data point is an average of five repeated analyses measured over a period of five days, good linearity was obtained (R^2 = 0.9989). Table 1 summarizes the results of analyses obtained by analyses of the above samples. The repeatability of measurements was less then 8% (RSD) within an experimental series for all examined matrices. Good agreement with data obtained by reference HPLC/UV method was obtained.

Conclusions

DART-TOFMS technique was demonstrated to be suitable for accurate determination of caffeine in various beverages including coffee and tea infusions. The requirements on sample preparation are minimal: only dilution, sonication and internal standard addition are needed. The results of preliminary experiments have shown the potential of DART-TOFMS to detect also other regulated compounds in soft drinks (artificial sweeteners, acidulants, preservation agents, etc.).

Figure 3 Positive DART spectrum: diluted coffee infusion.

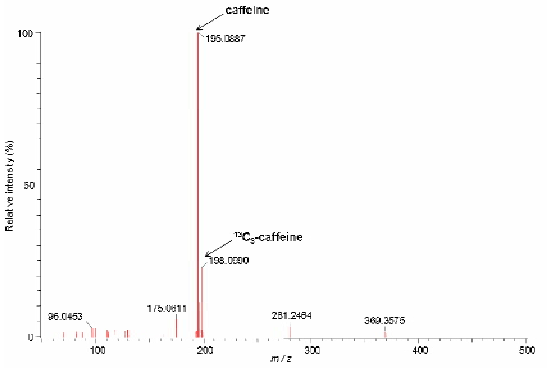

Figure 4 Calibration curve.

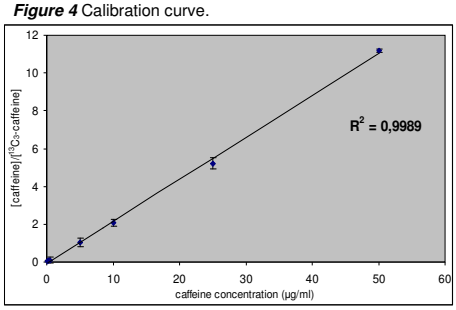

Table 1 Caffeine concentrations in examined samples.

Sample	Caffeine concentration (µg/ml)[a]	RSD (%)[b]
Coffee infusion	825	7.9
Black tea infusion	103	4.2
Green tea infusion	71	3.1
Ice tea	44	5.6
Cola drink	95	4.5
Energy drink	230	3.5

[a] calculated to undiluted beverage, [b] n = 5