

Rapid analysis of caffeine in various coffee samples employing direct analysis in real-time ionization–high-resolution mass spectrometry

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Abstract The development and use of a fast method employing a direct analysis in real time (DART) ion source coupled to high-resolution time-of-flight mass spectrometry (TOFMS) for the quantitative analysis of caffeine in various coffee samples has been demonstrated in this study. A simple sample extraction procedure employing hot water was followed by direct, high-throughput (<1 min per run) examination of the extracts spread on a glass rod under optimized conditions of ambient mass spectrometry, without any prior chromatographic separation. For quantification of caffeine using DART-TOFMS, an external calibration was used. Isotopically labeled caffeine was used to compensate for the variations of the ion intensities of caffeine signal. Recoveries of the DART-TOFMS method were 97% for instant coffee at the spiking levels of 20 and 60 mg/g, respectively, while for roasted ground coffee, the obtained values were 106% and 107% at the spiking levels of 10 and 30 mg/g, respectively. The repeatability of the whole analytical procedure (expressed as relative standard deviation, RSD, %) was <5% for all tested spiking levels and matrices. Since the linearity range of the method was relatively narrow (two orders of magnitude), an optimization of sample dilution prior the DART-TOFMS measurement to avoid saturation of the detector was needed.

Keywords Ambient mass spectrometry · Direct analysis in real time · Coffee · Caffeine

Introduction

Caffeine (3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione or 1,3,7-trimethylxanthine) is a biologically active xanthine alkaloid naturally occurring in coffee, tea, cacao, and guarana plant parts [1]. Among various types of beverages containing caffeine, coffee represents the traditional source for the adult population. The amount of caffeine in a cup of coffee can vary greatly, depending on the species and the origin of beans (Arabica has a lower caffeine content compared to Robusta), on the composition of the blend and on the method of brewing [2]. Since for some groups of consumers temporary changes in behavior (such as increased excitability, irritability, nervousness, or anxiety) were reported after consumption of caffeine-containing foods, the European Union enforced a Directive (2002/67/EC) dealing with the labeling of foodstuffs containing caffeine [3].

Several analytical approaches can be used for the analysis of caffeine in various beverages including coffee. In particular, instrumental techniques such as high-performance liquid chromatography (HPLC) coupled to either ultraviolet (UV) [4] or mass spectrometric (MS) detection [5], gas chromatography (GC) employing a flame ionization detector [6], thin-layer chromatography–mass spectrometry (TLC-MS) [7], capillary electrophoresis [8], Fourier transform infrared spectroscopy–attenuated total reflectance (FTIR-ATR) [9] have been employed. Considering the frequency of their use, the methods employing HPLC are dominating, not only because liquid chromatographs represent common laboratory equipment, but they also enable an

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easy operation with good performance characteristics for caffeine measurements.

In the recent years, a large number of novel ambient desorption ionization techniques, such as desorption electrospray ionization, atmospheric-pressure solids analysis probe, direct analysis in real time (DART), and many others have become available [10]. Their main advantages, compared to up-to-date conventional techniques (GC-MS, LC-MS), involve the possibility of direct sample examination in the open atmosphere, minimal or no sample preparation requirements (separation step not involved), and, remarkably high sample throughput. DART, which was investigated in this study, represents one of the APCI-related techniques employing a glow discharge for the ionization. Metastable helium atoms, originating in the plasma, react with ambient water, oxygen, or other atmospheric components to produce the reactive ionizing species [11]. The DART ion source has been shown to be efficient for soft ionization of a wide range of both polar and non-polar compounds. Until now, several papers have been published describing various DART applications including rapid analysis of various substances occurring in foodstuffs and food crops [12–17].

In this study, the challenge to develop a rapid method for a specific determination of caffeine in coffee using ambient mass spectrometry has been addressed. A DART ion source coupled to a high-resolution time-of-flight mass spectrometer has been investigated for this purpose. In addition, a comparison of the developed method with a conventional technique represented by HPLC-UV is provided.

Materials and methods

Chemicals

HPLC-grade methanol was purchased from Merck (Darmstadt, Germany). Water used for extractions was purified with a Milli-Q purification system (Millipore, Eschborn, Germany). The solutions for calibration curve preparation were made of caffeine and $^{13}\text{C}_3$ -caffeine diluted in an ethanol–water mixture (4:1, v/v; Sigma–Aldrich, Steinheim, Germany). Acetic acid for HPLC mobile phase was supplied by Lach-Ner (Neratovice, Czech Republic). Polyethylene glycol for mass drift compensation was from Sigma-Aldrich, (Steinheim, Germany).

Samples

Samples of roasted ground coffee ($n=7$), instant coffee ($n=14$), and capsuled coffee ($n=2$), representing a wide range of coffee products, were purchased from retail market and stored in dark and dry at 6 °C. Coffee beverages were prepared at the day of analysis, but no significant changes in caffeine

content were observed after 7 days of storage of the liquid samples in an airtight glass vessel at 6 °C.

Sample preparation

A simple extraction of caffeine from 1 g of dry roasted ground coffee, using 100 mL of boiling deionized water was carried out. For different ways of coffee beverages preparation, home devices were used: (a) Rowenta ES 4400 for espresso; (b) KRUPS KP5009E2 Dolce Gusto Circolo and (c) Bosch Tassimo TAS4041EE for capsuled coffee, and (d) Krups 183 for filtered coffee.

In the cases of espresso, filtered, and “mud coffee,” 7 g of roasted ground coffee was used. To get “mud coffee,” 100 mL of boiling water was added, in the cases of espresso and filtered coffee 75 mL of brew was obtained. Beverages made of instant coffee were prepared according to the recommendations of coffee producers, which means the addition of 150 mL of boiling water to 1.66 g of dry sample. Each infusion was filtered using a syringe filter (0.45 μm) before the analysis.

An important part of sample preparation before DART-TOFMS analysis was sample dilution. In the first step, the liquid samples were diluted with deionized water (a) 20 times for the extract of roasted ground coffee (prepared from 1 g of solid sample and 100 mL of water), (b) 100 times for “mud coffee,” espresso, and capsuled coffee brews and (c) ten times for instant coffee brew (prepared from 1.66 g of the sample and 150 mL of water). The second dilution was performed with HPLC-grade methanol. In this step, all the extracts were further ten times diluted. For a comparative HPLC-UV method, only the respective water dilution of the extracts was performed.

Quantification of caffeine in the extracts

For quantification of caffeine using DART-TOFMS, an external calibration in the range of 0.1–10 $\mu\text{g/mL}$ was used. An addition of isotopically labeled caffeine (100 μL of solution of $^{13}\text{C}_3$ -caffeine; 10 $\mu\text{g/mL}$) at a constant level (i.e., 1 $\mu\text{g/mL}$) to 1 mL of both, the sample extracts and the calibration solutions was used to compensate the variations of the ion intensities of caffeine signal during the measurements. For quantification of caffeine using HPLC-UV, an external calibration in the range of 1–250 $\mu\text{g/mL}$ was used.

Instrumentation and testing conditions

For the experiments, DART-TOFMS system consisting of a DART ion source (IonSense, Saugus, MA, USA), an Accu-TOF LP high-resolution time-of-flight mass spectrometer [JEOL (Europe), SAS, Croissy sur Seine, France] and an HTC PAL autosampler AutoDART-96 (Leap Technologies, Carrboro, NC, USA), was used.

The operating conditions of a DART ion source were as follows: positive/negative ion mode; helium flow: 4.0 L/min; gas beam temperature: 250 °C; needle voltage: 4.0 kV; perforated and grid electrode potentials: +150/–150 V and +250/–350 V, respectively. Conditions of TOFMS: monitored mass range, m/z 100–1,000; peaks voltage, 900 V; acquisition rate, 5 spectra/s; resolving power, approx. 6,000 FWHM (full width at half maximum); detector voltage, 2,400 V. The distance between the DART gun exit and mass spectrometer inlet was 10 mm. Sample introductions ($n=2$) were carried out automatically using Dip-it samplers (IonSense, Saugus, MA, USA). The sampling glass rod was immersed for 1 s into the sample hole of a deep-well micro-plate (Life Systems Design, Merenschwand, Switzerland) containing approx. 600 μL of respective sample, and transferred to the optimized position in front of the DART gun exit. The sample was then desorbed from the glass rod surface within 5 s, while the spectral data were recorded. To perform a mass drift compensation for accurate mass measurements and elemental composition calculations, a polyethylene glycol (average relative molecular weight 600) 200 $\mu\text{g}/\text{mL}$ solution in methanol, was introduced manually at the end of each sequence of samples.

HPLC-UV analysis was performed on an Agilent liquid chromatograph system (HP 1200, Agilent, USA) equipped with a DAD detector. A C18 reversed-phase packed column LiChroCART (125 \times 4.6 mm, 5 μm), equipped with a guard column LiChroCART (4 \times 4 mm) was used for separation throughout this study and the caffeine peak was detected at a wavelength of 274 nm. A gradient elution was performed by varying the proportion of solvent A (water–methanol–acetic acid, 90:8:2, $v/v/v$) to methanol, from 10% solvent A in the first 3 min to 100% methanol in the 11th minute. Reconditioning of the HPLC system was carried out after each sample for 3 min. A constant flow of 0.6 mL/min was used. A volume of 20 μL was injected.

Data processing

The Mass Center software version 1.3 (JEOL, Tokyo, Japan) was used for data processing. Mass spectral data were obtained by averaging of the mass spectra recorded during the exposure of the sample to the DART gas beam; background ions were subtracted and a mass drift was corrected. Agilent ChemStation B.04.03 software was used when working with HPLC-UV system.

Results and discussion

Optimization of DART-TOFMS parameters

In the first part of our experiments, the relationship between the setting of various DART operating parameters and the

features of mass spectra generated under particular conditions was investigated. In general, helium beam temperature, flow rate, and (thermal) desorption time are typically the major parameters affecting DART ion formation and effectiveness of their transmission into MS [12].

Protonated molecules $[M+H]^+$ were obtained under conditions of positive DART ionization when analyzing standards of caffeine and $^{13}\text{C}_3$ -caffeine dissolved in an ethanol–water mixture (4:1, v/v). In the case of caffeine, the $[M+H]^+$ ion corresponded to an elemental composition of $[\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2+H]^+$, while that of $^{13}\text{C}_3$ -caffeine to $[^{12}\text{C}_5^{13}\text{C}_3\text{H}_{10}\text{N}_4\text{O}_2+H]^+$, each characterized by the characteristic isotope pattern.

The impact of gas beam temperature was monitored for temperatures 150, 200, 250, 300, and 350 °C. The temperature of 250 °C provided the highest responses for both analytes tested. Helium flow rates were also observed to have an influence on the DART-TOFMS responses of target analytes. This parameter was tested for 3.0, 3.5, 4.0, and 4.5 L/min. A helium flow of 4.0 L/min gave the highest responses for both analytes.

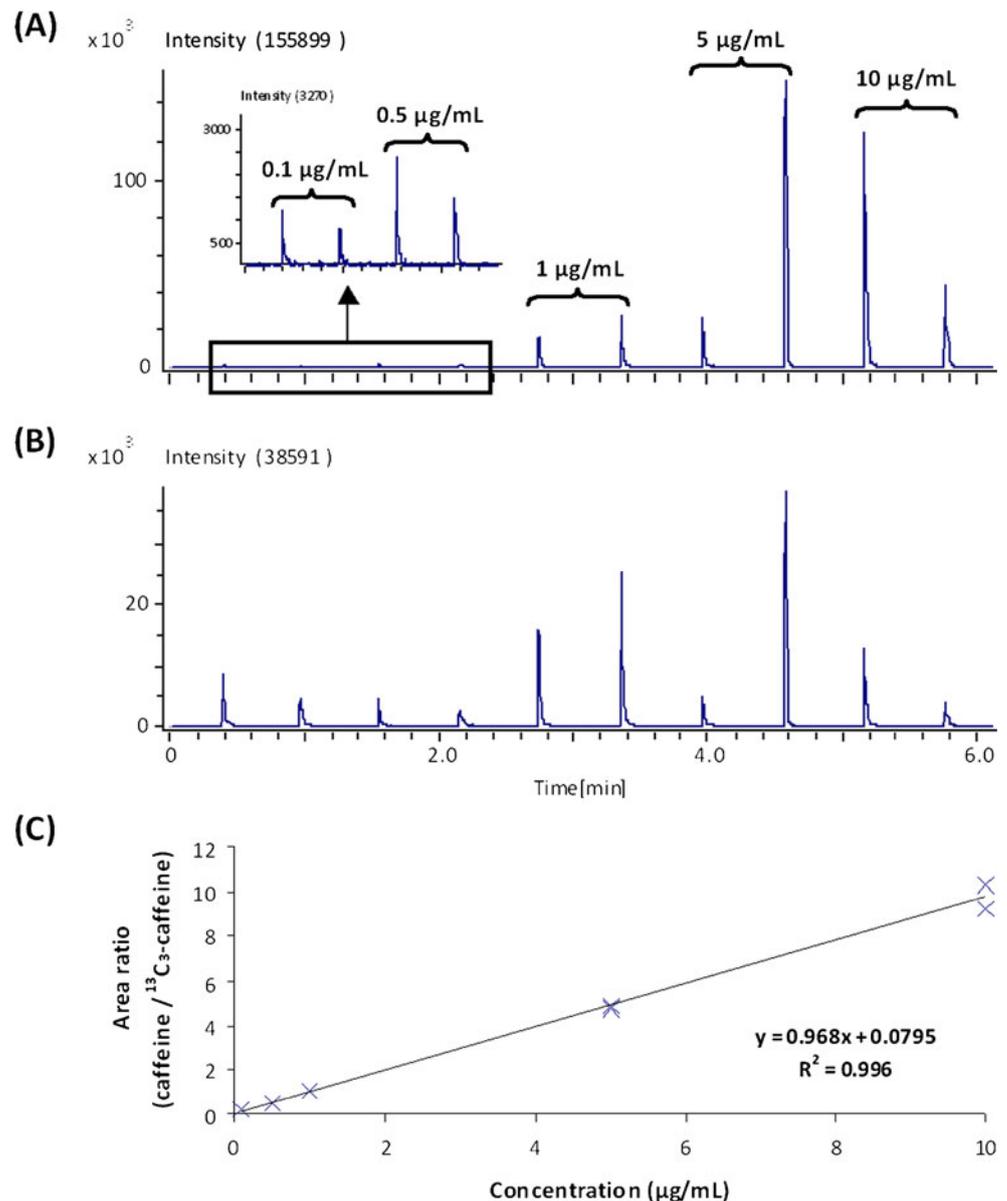
Another important factor optimized was (thermal) desorption time. To improve not only the throughput of analyses but also the quantitative DART measurements, a commercial autosampler device (AutoDART-96) was used. The robotic arm delivers the sample from a deep-well reservoir into the sampling region where desorption at fixed position occurs. In this way, compared to manual sampling, improvement of repeatability of the measurements was achieved. The tested values of (thermal) desorption time included 1, 2, 5, 10, and 30 s. It was observed that 5 s provided sufficient intensity of ions. Longer desorption time led only to a slight increase of intensities of some matrix co-extracts present in the sample extracts.

Analysis of coffee extracts

Once the DART parameters were optimized, the detection of caffeine in coffee extracts and beverages was investigated. Considering the differences in the caffeine content in the analyzed samples, dilution of both, the extracts and beverages needed to be optimized to obtain responses within a linear dynamic range of the TOFMS detector. The initial dilution of the extracts can be considered on the basis of the natural occurrence of caffeine in the examined samples since the content of caffeine in roasted coffee samples is typically in a relatively narrow interval, 0.8–2.5% for Arabica and up to 4% for Robusta. In the case of instant coffee, higher content of caffeine is typically observed (2.5–5.4%) [18].

During our experiments, we found that caffeine content in the examined extracts and beverages should be in the range of 0.1–10 $\mu\text{g}/\text{mL}$ to obtain good linearity of the calibration curve (Fig. 1). The intensity of the caffeine quantification ion was poorly reproducible as far as the

Fig. 1 DART-TOFMS analysis of calibration solutions containing caffeine and $^{13}\text{C}_3$ -caffeine (internal standard). **A** Repeated injections of caffeine (m/z 195.0882) in the concentration range 0.1–10 $\mu\text{g}/\text{mL}$; **B** Corresponding injections of $^{13}\text{C}_3$ -caffeine (m/z 198.0983) at a concentration of 1 $\mu\text{g}/\text{mL}$; **C** Calibration curve for caffeine demonstrating linearity in the concentration range tested



concentration was <0.1 $\mu\text{g}/\text{mL}$. On the other hand, saturation of the multi-channel plate (MCP) detector was observed with the caffeine content >10 $\mu\text{g}/\text{mL}$.

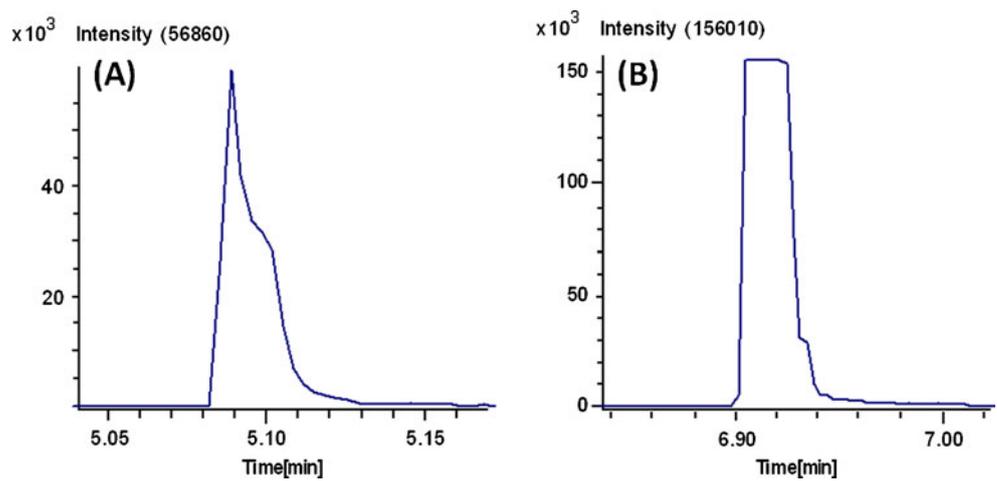
The saturation of the MCP detector is illustrated in Fig. 2, where the top of the MS peak is cut-off, in consequence of which the underestimation of peak area/height was registered. In fact, the profile of the MS peak should always be checked (especially in quantitative DART-TOFMS analysis), and, as far as saturation is observed, the extract should be re-analyzed after its dilution.

On the basis of conducted analyses, the optimal dilutions of the extracts for DART-TOFMS measurements were (a) 200 times for roasted ground coffee extract, (b) 1,000 times for “mud coffee,” espresso and capsuled coffee brews, and (c) 100 times for beverages prepared from dry instant coffee.

For quantitative analysis using a DART ion source, it is recommended to use an internal standard to compensate variation of the ion intensities of analytes [13]. Isotopically labeled internal standard ($^{13}\text{C}_3$ -caffeine) was used for this purpose. Although this internal standard is quite expensive (the price of native caffeine vs. $^{13}\text{C}_3$ -caffeine is 0.4 vs. 100 €), its use represents the best way for compensation of both, the variation of the ion intensities and the matrix effects due to the possible signal suppression/enhancement.

It should be noted that not only caffeine was detected in the coffee extracts, but also other polar compounds isolated during the extraction. Figure 3 shows the example of the DART-TOFMS records (measured in both positive and negative ion modes) of the roasted ground Arabica and Robusta

Fig. 2 Illustration of the saturation of the MCP detector (caffeine, m/z 195.0882, as an example). **A** Peak within the linear range of the MCP detector (1 $\mu\text{g/mL}$); **B** Peak outside the linear range of the MCP detector (100 $\mu\text{g/mL}$)



coffee brews. Thanks the exact mass measurement of TOFMS and estimation of elemental composition, a tentative identification of some sample components was possible. Although preliminary, these complex fingerprints might be considered in coffee authenticity assessment. Such an approach has been successfully used as a tool for authentication of beers [14], olive oils [13], and animal fats [19]. Of course, large sets of samples (covering different varieties of

coffee as well as geographical origin) would be required for the analysis to confirm this hypothesis.

DART-TOFMS method performance

In the follow-up part of the study, we investigated the recoveries of caffeine from instant and roasted ground coffee. For the experiments, decaffeinated instant and roasted

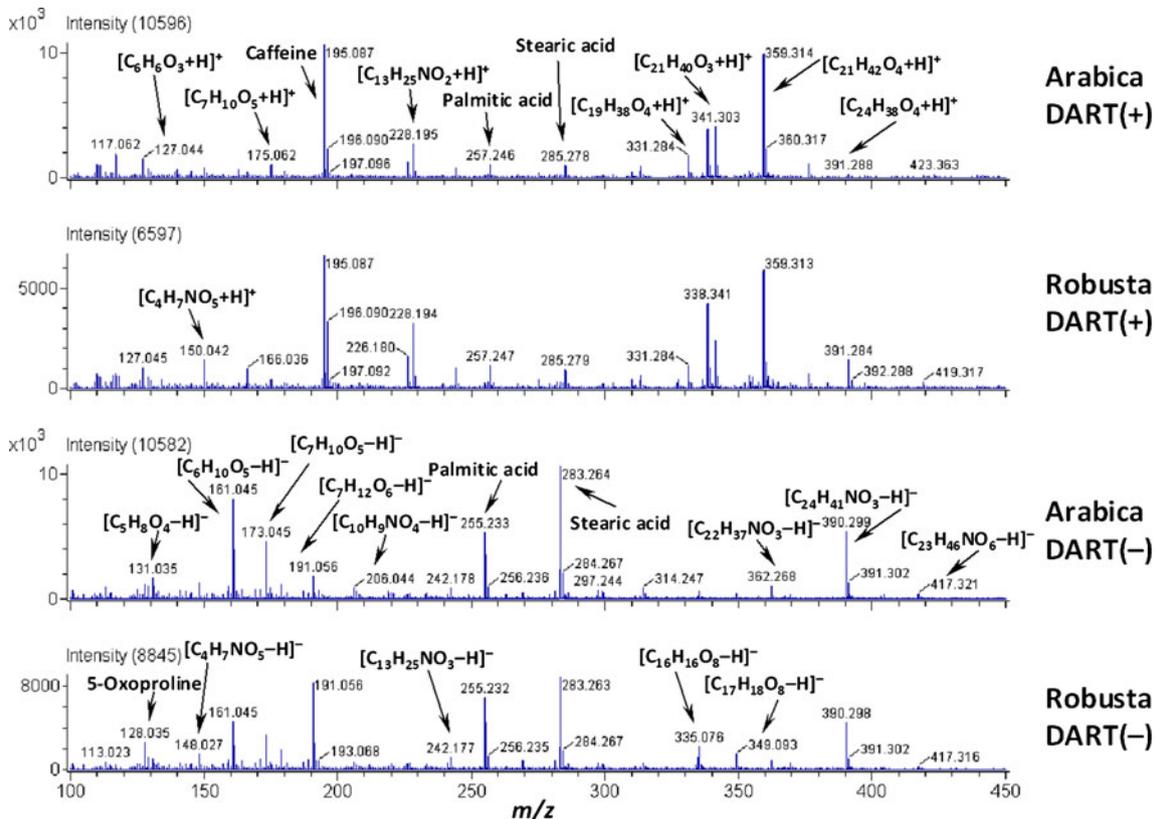
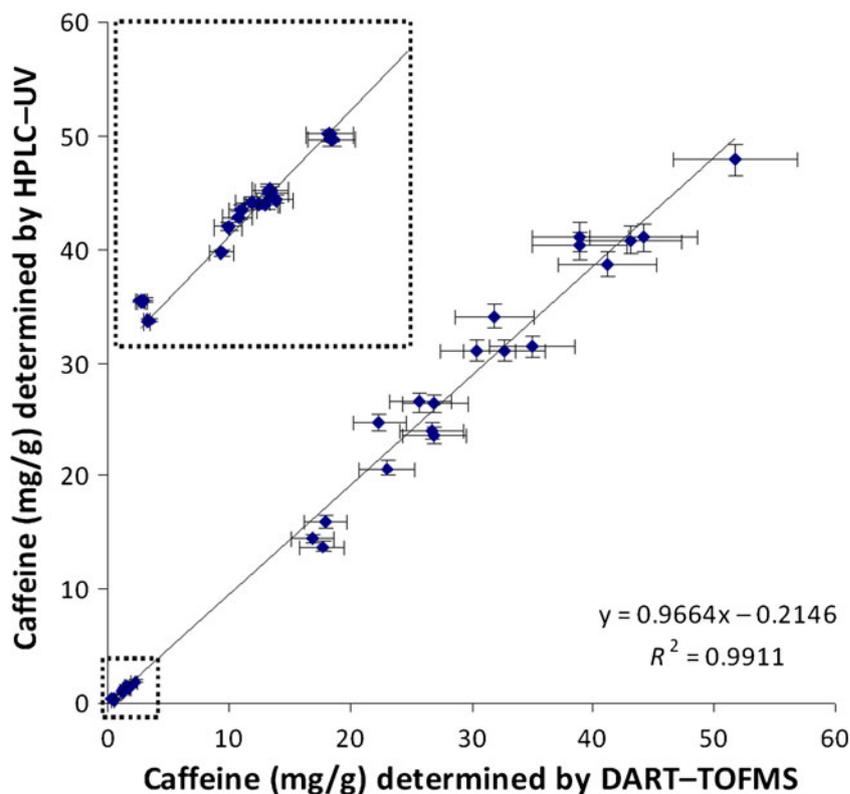


Fig. 3 DART-TOFMS spectra (m/z 100–450) acquired in DART positive and negative ion modes of the extracts of roasted ground coffee brew: **a** Arabica, **b** Robusta

Fig. 4 Correlation between caffeine contents (in milligrams per gram) determined by DART-TOFMS and that determined by HPLC-UV techniques in instant and roasted ground coffee samples ($n=21$); data for beverages ($n=23$) prepared from roasted ground coffee as espresso and “mud coffee,” and for beverages prepared from capsule coffee, expressed in milligrams per milliliter)



ground coffee samples were used. Five replicates were carried out for each spiking level. Recoveries of the DART-TOFMS method were 97% for instant coffee at the spiking levels of 20 and 60 mg/g, respectively, while for roasted ground coffee, the obtained values were 106% and 107% at the spiking levels of 10 and 30 mg/g, respectively. The repeatability of the whole analytical procedure (expressed as relative standard deviation, RSD, %) was <5% for all tested spiking levels and matrices.

Comparison of DART-TOFMS and HPLC-UV results

To confirm the validity of the DART-TOFMS results, we performed side-by-side HPLC-UV analyses of 23 different coffee sample extracts (prepared in replicates). Figure 4 compares the results that show a good correlation between the analyses (both the slope and correlation coefficient are close to 1).

In general, the amount of caffeine in analyzed roasted ground coffee samples was in the range of 16.9–26.9 mg/g while in instant coffee samples in the range of 22.4–51.7 mg/g. Beverages prepared from roasted ground coffee as espresso and “mud coffee,” and beverages prepared from capsuled coffee contained 0.5–2.2 mg/mL of caffeine while the levels in instant coffee brews were—due to a smaller coffee/water ratio—lower (0.4–0.5 mg/mL).

Conclusions

High-throughput, fully automated quantitative analysis of caffeine in various coffee samples was performed following a simple extraction procedure using a DART ion source coupled to high-resolution TOFMS. Optimized DART-TOFMS procedure enables the detection of caffeine at levels $>0.1 \mu\text{g/mL}$ (extract). A good agreement of the DART-TOFMS and HPLC-UV results was obtained for various coffee samples; however, thanks to the 14-times increased sample throughput (time for instrumental analysis considered), the DART-TOFMS represents a considerably quicker option.

It should be noted that a DART ion source can be attached to most common mass spectrometers on the market; the only operation required is disconnection of an LC unit. With regard to the low cost of this ion source as compared to MS instruments, we presume that ambient mass spectrometry will find a lot of routine applications in all laboratories requiring high-throughput measurements.

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