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Caffeine in Your Drink: Natural or Synthetic?

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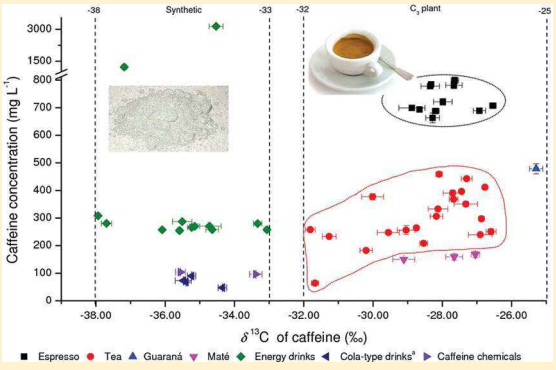
Supporting Information

ABSTRACT: Owing to possible adulteration and health concerns, it is important to discriminate between natural and synthetic food ingredients. A new method for compound-specific isotope analysis (CSIA) by coupling high-temperature reversed-phase liquid chromatography to isotope ratio mass spectrometry (HT-RPLC/IRMS) was developed for discrimination of natural and synthetic caffeine contained in all types of drinks. The analytical parameters such as stationary phase, column inner diameter, and column temperature were optimized for the separation of caffeine directly from drinks (without extraction). On the basis of the carbon isotope analysis of 42 natural caffeine samples including coffee beans, tea leaves, guaraná powder, and maté leaves, and 20 synthetic caffeine samples from different sources by high-temperature reversed-phase liquid chromatography coupled to isotope ratio mass spectrometry, it is concluded that there are two distinguishable groups of caffeine δ^{13}C-values: one between −25 and −32‰ for natural caffeine, and the other between −33 and −38‰ for synthetic caffeine. Isotope analysis by HT-RPLC/IRMS has been applied to identify the caffeine source in 38 drinks. Four mislabeled products were detected due to added but nonlabeled synthetic caffeine with δ^{13}C-values lower than −33‰. This work is the first application of HT-RPLC/IRMS to real-world food samples, which showed several advantages: simple sample preparation (only dilution), high throughput, long-term column stability, and high precision of δ^{13}C-value. Thus, HT-RPLC/IRMS can be a very promising tool in stable isotope analysis of nonvolatile compounds.



For manufacturers and customers, it is interesting to discriminate between natural and synthetic products, especially for widely consumed food products like caffeine-containing drinks. Caffeine-containing drinks are the most popular type of beverage in the world.[1] Apart from natural drinks such as coffee, tea, guaraná, and máte, caffeine is also found in energy drinks and cola-type soft drinks that usually contain added synthetic caffeine. However, people prefer food products made of natural sources to those made of artificial chemicals. The food and drug administration (FDA) regulates that caffeine must be listed on the label of drinks when it has been added in the production, but not for drinks made from tea or coffee.[2] In consideration of the growing demand for natural drinks on the one hand and the significant price differences between naturally occurring caffeine sources and synthetic caffeine chemicals on the other hand, there is a high risk of fraud by false declaration of caffeine origins. Moreover, the naturally caffeinated drinks are generally assumed to be healthier than energy drinks containing high levels of synthetic caffeine[1,3−5] that can lead to adverse effects, such as anxiety and insomnia.[6,7] Some energy drinks contain caffeine in excess of 400 mg, which is the maximum daily allowance of caffeine for a healthy adult.[7,8] Discrimination of natural and synthetic caffeine has received attention since energy drinks first appeared in Europe and Asia in 1960s when radiocarbon analysis of caffeine was used for identification as to its natural or synthetic origin.[9,10]

Stable isotope analysis has proved to be a powerful tool for detecting adulteration in food products.[11−15] The carbon stable isotope ratio depends upon the origin of the material. For example: plants using the C_3-photosynthetic pathway have more negative δ^{13}C-values than those using the C_4 pathway. Commercial chemicals derived from petroleum and coal sources may have different δ^{13}C value compared to those extracted from biogenic sources.[16] Richling et al. report that an elemental analyzer coupled to isotope ratio mass spectrometry (EA/IRMS) for δ^{13}C and δ^{18}O analysis has the potential to discriminate between natural and synthetic caffeine.[17] However, both reported methods, radiocarbon analysis and EA/IRMS, need off-line extraction and purification of caffeine from the matrix, which is a labor-intensive and time-consuming process. Liquid chromatography coupled to isotope ratio mass spectrometry (LC/IRMS) has gained growing interest as it is able to measure carbon stable isotope ratios of single compounds directly from complex mixtures.[18,19] It has been applied successfully to authenticity control of ethanol in wine[20,21] and sugars in honey.[20,22] The recent introduction of high-temperature liquid chromatography coupled to isotope ratio mass spectrometry (HT-RPLC/IRMS) now enables the

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Table 1. Measured Caffeine Samples and Preparation Methods[a]

type	sample	total	EA/IMRS	LC/IRMS
natural sources	coffee beans (Arabic)	18	see ref 26	espresso was prepared using an espresso machine of Saeco Royal Coffee Bar (Essen, Germany), and then diluted 5 times and filtered through a 0.20 μm membrane filter[37]
	tea leaves	21	see ref 32	1 g finely grinded tea leaves in 100 g water was boiled for 20 min, then diluted 2 times and filtered through a 0.20 μm membrane filter
	guaraná	1	NA	same as tea leaves
	maté	3	NA	same as tea leaves
synthetic caffeine	commercial chemicals	2	measured directly	100 mg L^{-1}
	energy drinks[b]	18	see ref 26	diluted 5 times and filtered through a 0.20 membrane filter
tested drinks	bottled or canned drinks	38	NA	diluted (if necessary)[c] and filtered through a 0.20 membrane filter

[a]Except for two caffeine chemicals (Fluka, Steinheim Germany; Alfa Aesar, Karlsruhe, Germany), the samples were collected from a local market. [b]For energy drinks that contain synthetic caffeine, it is typical to see that an exact amount of caffeine is listed on the product label and no information about a natural caffeine source is given. [c]Samples with caffeine concentrations above 200 mg L^{-1} were diluted in order to achieve a caffeine concentration of 100 mg L^{-1}, approximately.

use of reversed-phase columns for compound-specific isotope analysis.[23,24]

In this work, HT-RPLC/IRMS was developed for carbon isotope measurements of caffeine directly from drinks. The determined $\delta^{13}C$ values can be used as a tool for discrimination of natural and synthetic caffeine based on two distinct ranges of caffeine isotope ratios as demonstrated by analyzing 42 natural caffeine samples including coffee, tea, guaraná, and maté and 20 caffeine samples of varying synthetic origin.

EXPERIMENTAL SECTION

Chemicals. Ortho phosphoric acid (99%) and sodium peroxodisulfate (99%) were purchased from Fluka (Buchs, Switzerland). Caffeine (99%) was purchased from Fluka (Steinheim, Germany). Theophylline monohydrate (99%) and theobromine (99%) were purchased from Alfa Aesar (Karlsruhe, Germany). Acetanilide (internal laboratory standard for elemental analysis) was purchased from Merck (Darmstadt, Germany). IAEA-600 caffeine with a $\delta^{13}C$ value of −27.77 ± 0.04‰ was purchased from International Atomic Energy Agency (Vienna, Austria).[25] Deionized water was used for solution preparation and mobile phase. Water and solutions used in the interface were degassed in an ultrasonic bath (Bandelin Electronic, Berlin, Germany) for 15 min under vacuum conditions. A MZ2D NT diaphragm pump (Vacuubrand, Wertheim, Germany) was used to generate the needed vacuum. After degassing, it was continuously purged with Helium 5.0 (Air Liquide, Oberhausen, Germany).

The measured caffeine samples and preparation method for EA/IRMS and HT-RPLC/IRMS measurements are listed in Table 1. Prior to EA/IRMS measurement, the purity of isolated caffeine from coffee beans, tea leaves, and energy drinks was checked by a system 2000 Fourier transform infrared spectrometer (PerkinElmer, Rodgau, Germany) and by high performance liquid chromatography. In order to check for potential isotope fractionation in the sample preparation procedure of the EA/IRMS measurement, the synthetic caffeine sample with a known $\delta^{13}C$ value was subjected to the same sample procedure as coffee and tea samples and subsequently measured by EA-IRMS.[26]

Instrumentation for HT-RPLC/IRMS. The eluent for liquid chromatography was delivered by a Rheos Allegro pump (Flux instruments AG, Basel, Switzerland). The injection was made into a 10 μL sample loop using a HTC PAL autosampler (CTC Analytics, Zwingen, Switzerland). An HT-HPLC 200 column oven (SIM, Oberhausen, Germany) was used for mobile phase preheating and for heating the column with two aluminum blocks. For isotope ratio measurement, a LC-IsoLink interface (Thermo Fisher Scientific, Bremen, Germany) connecting the HT-HPLC oven with a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific) was used. The water flow rate was 0.5 mL min^{-1}. The flow rate of sodium peroxodisulfate (0.83 mol L^{-1}) and phosphoric acid (1.50 mol L^{-1}) was 50 μL min^{-1} each. In order to avoid blockage in the system, an in-line filter with a pore size of 0.5 μm (Vici, Schenkon, Switzerland) was placed in front of the oxidation reactor of the LC-IsoLink interface. The schematic setup is the same as shown in Figure 1 of ref 23. Without the column, the setup may be used for flow injection analysis (FIA-IRMS).[27]

For the separation of caffeine from drinks, an XBridge C_{18} column (2.1 × 100 mm, 3.5 μm, Waters, Eschborn, Germany) with an XBridge C_{18} guard column (2.1 × 10 mm, 3.5 μm) was used. Two further columns (XBridge C_{18} (3.0 × 100 mm, 3.5 μm) and Zirchrom PBD (3.0 × 150 mm, 5 μm, Zirchrom, Anoka, USA)) were used for method development.

Isotopic Calculation. All reported isotope ratios are expressed as $\delta^{13}C$ values relative to the international VPDB standard (Vienna Pee Dee Belemnite). $\delta^{13}C$ is defined as

$$\delta^{13}C_{s,VPDB} = \frac{R(^{13}C/^{12}C)_s}{R(^{13}C/^{12}C)_{VPDB}} - 1 \quad (1)$$

In the equation, $R(^{13}C/^{12}C)_s$ and $R(^{13}C/^{12}C)_{VPDB}$ (0.0111802)[28] are the $^{13}C/^{12}C$ ratio in the sample and in the standard, respectively. At the beginning of each run, three pulses of a laboratory standard gas ($\delta^{13}C$ = −37.81‰) were introduced. The second peak was used for $\delta^{13}C$ calibration. Furthermore, another pulse was set at the end of the chromatographic run for controlling $\delta^{13}C$ consistency (see Figure 1). All data acquisition, processing, and evaluation were carried out using Isodat 2.5. The background subtraction was made automatically by Isodat 2.5; the background type used for peak integration was "individual background" with identical start and end slopes of 0.5 mV s^{-1} for most of the chromatograms.

According to the suggestion of Paul et al.,[29] a two-point normalization was applied to correct the offset in $\delta^{13}C$ values measured by HT-RPLC/IRMS. The method uses a linear regression of measured $\delta^{13}C$ values ($\delta_{M,std1}$;$\delta_{M,std2}$) and true

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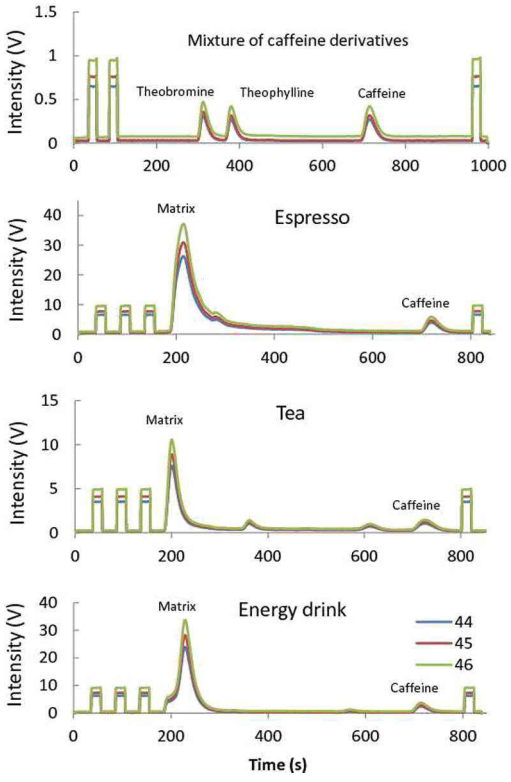

Figure 1. HT-RPLC/IRMS chromatograms of a mixture of caffeine derivatives (100 mg L^{-1}), espresso, tea, and energy drink sample. The temperature used was 80 °C and the column was a XBridge C18 (2.1 × 100 mm, 3.5 2 m). The second reference gas peak was used for calibration of δ ^{13}C-values.

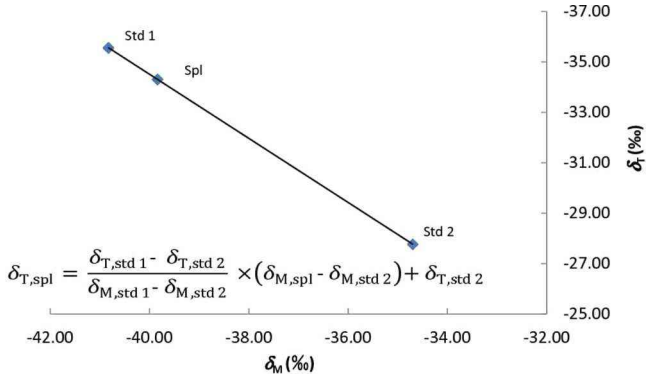

Figure 2. Illustration of the derivation of eq 2 used for two-point normalization of measured δ^{13}C by LC/IRMS. Two internal laboratory standards of caffeine samples (100 mg L^{-1}) with δ^{13}C of −27.77‰ and −35.56‰ were measured along with the samples by HT-HPLC/IRMS.

δ^{13}C -values ($\delta_{T,std1}$;$\delta_{T,std2}$) of two internal laboratory standards as shown in Figure 2. The δ^{13}C measured by HT-RPLC/IRMS ($\delta_{M,spl}$) was normalized by the following equation:[29]

$$\delta_{T,spl} = \frac{\delta_{T,std1} - \delta_{T,std2}}{\delta_{M,std1} - \delta_{M,std2}} \times (\delta_{M,spl} - \delta_{M,std2}) + \delta_{T,std2} \quad (2)$$

On the basis of the identical treatment (IT) principle for referencing isotope analysis,[28] two internal laboratory standards of caffeine chemicals at a concentration of 100 mg L^{-1} were measured along with the samples by HT-RPLC/IMRS, since they are chemically identical to the targeted compound and have δ^{13}C values of −27.77 and −35.56‰, which bracket the isotope ratio of most unknown samples.[25]

EA-IRMS Measurement. δ^{13}C values of pure compounds were measured with an EA 1110 Elemental Analyzer (CE instrument, Milan, Italy) coupled to a MAT 253 IRMS (Thermo Fisher Scientific) via a ConFlo IV interface (Thermo Fisher Scientific). In order to obtain the corrected $\delta^{13}C_{EA\text{-}IRMS}$ value of a compound, the δ^{13}C value measured by EA-IRMS was converted to the VPDB scale by using a spreadsheet evaluation as recommended by Werner and Brand.[28] More detailed information can be found in ref 23 and 28.

RESULTS AND DISCUSSION

Baseline Separation of Caffeine by HT-RPLC/IRMS. For compound-specific isotope analysis, baseline separation of target compounds from other components in the matrix is a key factor for achieving precise and accurate δ^{13}C. On the basis of a previous study, three different thermostable columns of two XBrige C_{18} columns and one Zirchrom PBD were selected to develop the method for the separation of caffeine from various mixtures including espresso, tea, and energy drink.[23] At a temperature of 80 °C the XBridge C_{18} column with inner diameter (i.d.) of 2.1 mm was able to fully separate caffeine from the mixtures within 15 min (see Figure 1). The analytical method was fast and simple. None of samples required any prepurification or pre-enrichment procedure. After dilution and filtration, they were directly injected. The background of the sample matrix did not affect the caffeine peak, and no further coeluted peak was observed during separation. Moreover, the carryover from late-eluting residue of the preceding samples did not affect subsequent measurements during sequence analysis. A guard column with the same stationary phase of C_{18} was used for the separation. This can offer protection by trapping unwanted components that would otherwise be retained strongly on the analytical column. In one instance, carryover was observed during day-to-day measurements. In this case, the guard and analytical columns were cleaned by flushing with pure methanol and regenerated by purging with water at 80 °C. In order to check whether the generally coexisting caffeine derivatives in coffee and tea can affect the separation of caffeine, a mixture of theobromine, theophylline, and caffeine had been measured under the same conditions. As shown in Figure 1, theobromine and theophylline eluted much earlier than caffeine without coelution.

Another XBridge C_{18} with an inner diameter of 3.5 mm was also able to baseline resolve caffeine from different mixtures mentioned above. However, the analysis time was 3 min longer than on the smaller column with an inner diameter of 2.1 mm while other separation parameters were the same. The optimum flow rate for the 2.1 mm i.d. column at a temperature of 80 °C was reported to be 0.5 mL min^{-1}.[24,30] This meets the requirement of the LC−IsoLink interface very well since the total flow of mobile phase, acid reagent, and oxidant reagent must be lower than 0.7 mL min^{-1}. A Zirchrom PBD column was also used for caffeine separation. However, this column was not able to baseline resolve caffeine in real-life samples. Therefore, the XBridge C_{18} with 2.1 mm i.d. was chosen for the application of caffeine isotope analysis by HT-RPLC/IRMS.

Increasing the temperature can shorten analysis time of caffeine on the XBridge C_{18} column due to the improved elution strength of water at elevated temperature.[23] However,

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using lower temperature can certainly prolong the life of the column by reducing the possibility of stationary phase degradation. Here we used a maximum temperature of 80 °C for XBridge C_{18} column as recommended by the manufacturer. Under these conditions, the column has been used for approximately 800 injections in four months. After accomplishing all measurements in this work, it still had a good performance. Such a long-term stability renders the method usable for routine analysis. Furthermore, the long lifetime of the column in this application recommends its use also in high-temperature liquid chromatography with pure-water mobile phases.

Precision and Accuracy of Caffeine $\delta^{13}C$. For a reliable isotope analysis, $\delta^{13}C$ values must be determined with high accuracy and precision. The precision of the developed method was tested in the concentration range of caffeine from 20 to 400 mg L^{-1}. At each concentration level triplicates were measured. In the concentration range from 400 to 30 mg L^{-1}, the standard deviation (SD) for triple measurement at each concentration are less than 0.22‰ and the $\delta^{13}C$ value at each concentration is within ±0.5‰ (see Figure 3). Furthermore, there is a good

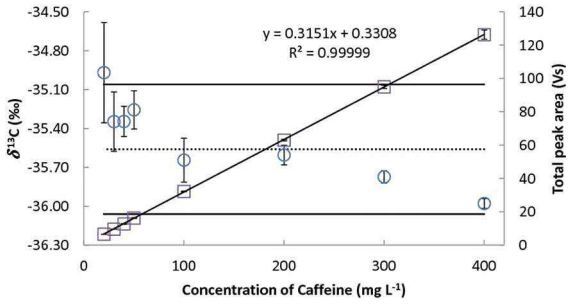

Figure 3. $\delta^{13}C$ values of caffeine in the concentration range from 20 to 400 mg L^{-1}. The circles and squares represent the $\delta^{13}C$ and total peak area, respectively. The linear curve fit and the correlation coefficient for plotting peak area vs concentration are shown in the graph. Error bars indicate the standard deviation of triplicate measurements. The dotted line indicates the iteratively calculated mean value of $\delta^{13}C$. The horizontal solid lines represent the interval of mean value ±0.5‰[30].

linear relationship between the total peak area and caffeine concentration (R = 0.99999). Therefore, not only precise $\delta^{13}C$ values but also caffeine concentrations can be obtained in this concentration range. At a concentration of 20 mg L^{-1}, the $\delta^{13}C$ value is outside ±0.5‰. Therefore, 30 mg L^{-1} is the detection limit of caffeine isotope analysis by HT-RPLC/IRMS according to the approach of defining the detection limit for compound-specific isotope analysis.[31]

The accuracy of $\delta^{13}C$ values obtained by the developed method was evaluated by comparison with $\delta^{13}C_{EA/IRMS}$ values which were obtained by EA-IRMS analysis of isolated pure caffeine samples from different sources. It is known that there is an offset between the $\delta^{13}C$ values of caffeine measured by HT-RPLC/IRMS and true values (see Figure 2) due to the incomplete oxidation of caffeine in the LC–IsoLink interface.[23] Therefore, a procedure of two-point normalization was used to correct the $\delta^{13}C$ values measured by HT-RPLC/IRMS ($\delta_{M,spl}$) as described in the Experimental Section. Via the correction, the obtained $\delta^{13}C$ vaules ($\delta_{T,spl}$) are consistent with $\delta^{13}C_{EA/IRMS}$ values, proving acceptable accuracy (Table 2). The differences between $\delta_{T,spl}$ and $\delta^{13}C_{EA/IRMS}$ of caffeine in complex mixtures of espresso, tea, and energy drinks are lower than 0.43‰. The

Table 2. Comparison of Corrected $\delta^{13}C$ Values of Caffeine from Various Sources Measured by HT-RPLC/IRMS ($\delta_{T,spl}$) with EA/IRMS Analysis Results

caffeine source	$\delta_{T,spl}$ ± SD (n = 3)	$\delta^{13}C_{EA/IRMS}$ ± SD (n = 3)	$\lvert\delta_{T,spl} - \delta^{13}C_{EA/IRMS}\rvert$
coffee beans 1	−28.19 ± 0.19	−28.23 ± 0.04	0.04
coffee beans 2	−27.98 ± 0.27	−28.19 ± 0.07	0.21
tea leaves 1	−31.27 ± 0.20	−30.92 ± 0.08	0.35
tea leaves 2	−30.20 ± 0.18	−29.77 ± 0.08	0.43
energy drink	−35.59 ± 0.06	−35.76 ± 0.03	0.17
pure chemical	−33.38 ± 0.18	−33.36 ± 0.08	0.02

$\delta_{T,spl}$ values of one pure caffeine chemical (−33.38 ± 0.18‰) are in good agreement with $\delta^{13}C_{EA/IRMS}$ results with a difference of 0.02‰. The insignificant differences between $\delta_{T,spl}$ and $\delta^{13}C_{EA/IRMS}$ results indicate that reliable $\delta^{13}C$ values of caffeine have been achieved using the two-point normalization in this work. Two external standards, which possess the same chemical identity as the target compound and have $\delta^{13}C$ values bracketing the $\delta^{13}C$ values of most unknown samples, could be helpful for achieving reliable $\delta^{13}C$ for compound-specific isotope analysis according to the literature,[25,29] especially when no suitable internal standard is available.

Distinguishable $\delta^{13}C$ Ranges of Natural and Synthetic Caffeine. Two distinguishable $\delta^{13}C$ ranges of natural and synthetic caffeine were found based on the measurements of 42 natural caffeine samples including espresso, tea, maté, and guaraná from various geographic origins and measurements of 20 synthetic caffeine samples including energy drinks, cola-type drinks, and commercial chemicals (Figure 4). For drinks that

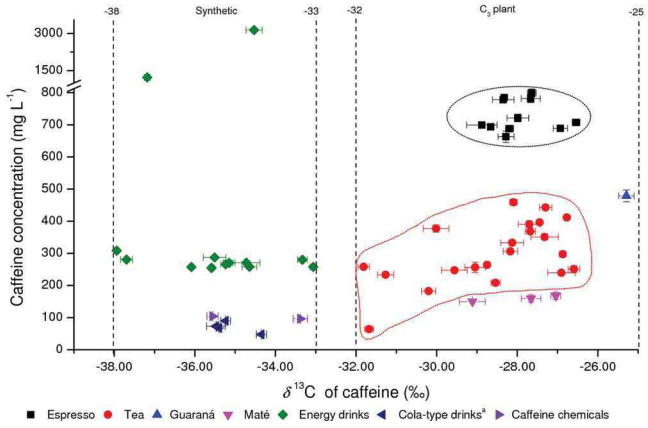

Figure 4. $\delta^{13}C$ values and concentrations of caffeine from different sources. Error bars indicate the SD of triplicate measurements. Four dashed lines represent two different ranges of $\delta^{13}C$ values, from −25 to −32‰ for natural caffeine in the C3-plant and from −33 to −38‰ for synthetic caffeine. (a) Cola-type drinks except for Coca Cola.

contain synthetic caffeine, it is typical to see that an exact amount of caffeine is listed on the product label and no information about a natural caffeine source is given. Figure 4 shows that caffeine extracted from natural sources of C_3-plants has $\delta^{13}C$ values between −25 and −32‰, while synthetic caffeine has more negative $\delta^{13}C$ values between −33 and −38‰.

Among a variety of natural drinks, the $\delta^{13}C$ value and content of caffeine in espresso ranged from −25.8 to −28.7‰ and from 663 to 950 mg L^{-1} (30–50 mg per cup in 52 mL), respectively (black squares in Figure 4). The espresso drinks were made

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from 18 coffee bean samples of different geographic origins distributed over East Africa, South America, Central America, and Oceania. According to several studies, caffeine $\delta^{13}C$ values of various coffee beans (50 samples) measured by EA/IRMS are between −25.1 and −29.9‰.[26,32,33] Our $\delta^{13}C$ values measured by HT-HPLC/IRMS are within the summarized range. The amounts of caffeine in various espresso drinks fit very well with the results (from 30 to 50 mg per cup) reported by the international food information council foundation.[34] In tea drinks, the caffeine $\delta^{13}C$ values and concentrations varied from −26.6 to −31.8‰ and from 64 to 459 mg L^{-1}, respectively. The collected 21 samples include four major types of tea: black, green, white, and Oolong; they originate from different countries including China, India, and Sri Lanka. The distribution of tea (red circles) and coffee caffeine (black squares) in Figure 4 indicates that ^{13}C of caffeine in some tea samples was more depleted than that in coffee samples, covering a wider isotopic range for tea samples. The same conclusion is true for other studies in the literature, which report $\delta^{13}C$ values of tea caffeine ranging between −27.2 and −32.4‰ (23 samples).[32,33] With these ranges it even becomes possible to differentiate between tea and coffee caffeine because tea caffeine but not coffee caffeine may have $\delta^{13}C$ values more negative than −30‰. Due to the difficulty in collecting maté leaves and guaraná seeds, only three maté and one guaraná samples were measured. Maté-caffeine $\delta^{13}C$ values and concentrations vary from −27.7 to −29.1‰ and from 159 to 168 mg L^{-1}, respectively (rose triangles in Figure 4). The caffeine $\delta^{13}C$ value of guaraná sample was −25.3‰ (blue triangles in Figure 4). The $\delta^{13}C$ range for maté and guaraná caffeine reported in the literature covers a range from −25.9 to −32.3‰ (five samples) and from −26.7 to −28.7‰ (32 samples), respectively.[17] The variation of caffeine $\delta^{13}C$ values in C_3-plants can be explained by the different extents of isotopic fractionation during photosynthesis caused by secondary external factors, such as humidity, temperature, and sunlight availability.[35,36]

Compared with natural caffeine, synthetic caffeine used in energy drinks has a more negative carbon isotope ratio, falling in a distinct group between −33.1 to −37.9‰. Most energy drinks contained caffeine with a concentration of approximately 300 mg L^{-1}, except for two brands ("quick energy", 3136 mg L^{-1}, and "energy shot", 1223 mg L^{-1}). The amount of caffeine found in various energy drinks is approximately the same as that declared on the label. The caffeine present in cola-type drinks varied in concentration ranging from 48 to 90 mg L^{-1}, which is much lower than that found in energy drinks. Caffeine in different cola drinks was between −34.4 and −35.5‰. Two commercial caffeines had $\delta^{13}C$ values of −33.4 and −35.6‰. The few literature data also report more negative $\delta^{13}C$ values for synthetic caffeine, ranging from −35.8 to −40‰ (seven samples).[26,32,33] The $\delta^{13}C$ variation in synthetic caffeine is probably due to different raw materials and synthesis pathways.

The results in this work corroborate the scarce information available in the literature and prove that $\delta^{13}C$ values for natural and synthetic caffeine can be distinguished. They fall into two distinct groups without significant overlap: from −25 to −32‰ for natural caffeine and from −33 and −40‰ for synthetic caffeine. This finding is based on a variety of natural caffeine samples with a large sample size (152 samples) and various synthetic caffeine samples (27 samples) that have been measured via two methodologies of LC/IRMS and EA/IRMS by different laboratories. If in the measurement of an unknown sample the observed $\delta^{13}C$ falls below the threshold of −32‰, it is assumed that the contained caffeine is synthetic, that is, not of natural provenience. The error probability for this assumption is $\alpha \approx 1\%$ (see the Supporting Information: setting a threshold for assigning an unknown caffeine sample to natural or synthetic provenience).

Authenticity Control of Caffeine-Containing Drinks. Identification of caffeine as either natural or synthetic is very important for authenticity control of caffeine-containing drinks. Naturally caffeinated drinks such as bottled or canned tea and instant coffee have expanded around the world. Some energy drinks use guaraná extracts to meet the customer's preference for natural products. According to the descriptions on the labels all 38 drinks in this study were supposed to contain natural caffeine. Sample preparation was very simple for all drinks; after dilution (if necessary) and filtration, it was directly injected and measured by HT-HPLC/IRMS. Subsequently, the obtained $\delta^{13}C$ values were used for identification of natural or synthetic origin based on the respective isotope ratio range.

As seen in Figure 5, four out of the 38 tested drinks contain caffeine with $\delta^{13}C$ values more negative than −33‰, falling

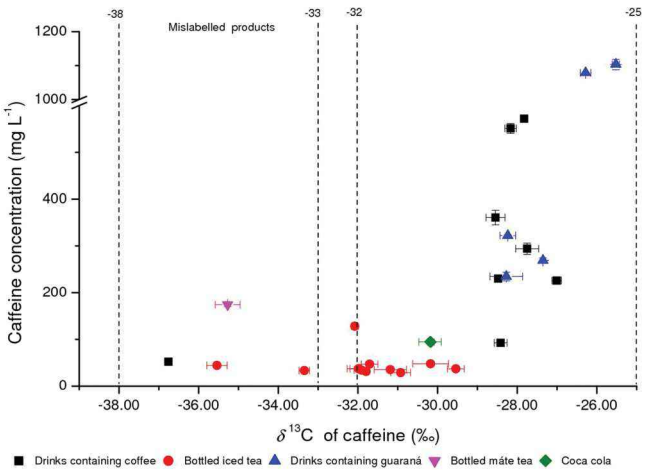

Figure 5. $\delta^{13}C$ values and concentrations of caffeine in various drinks that were supposed to contain natural caffeine sources according to the labels. Four products were found to be mislabeled. Error bars indicate the SD of triplicate measurements. Four dashed lines represent two different ranges of $\delta^{13}C$ values, from −25 to −32‰ for natural caffeine in C_3-plants and from −33 to −38‰ for synthetic caffeine.

into the group for synthetic caffeine. These four probably mislabeled drinks are one instant drink mix, two bottled iced tea drinks, and one maté drink. For six different instant coffee samples, the caffeine $\delta^{13}C$ values vary from −27.0 to −28.8‰, lying within the range found in coffee beans (from −25.1 to −29.9‰), and the caffeine concentrations are from 229 to 571 mg L^{-1}. One natural cola drink, which according to product description contained a coffee bean extract, has caffeine $\delta^{13}C$ value of −28.4‰ confirming a natural origin. However, one instant coffee had a $\delta^{13}C$ value of −36.8‰, indicating a synthetic origin. Twelve drinks of bottled tea contain caffeine with concentrations and $\delta^{13}C$ values ranging from 30 to 128 mg L^{-1} and from −29.6 to 31.9‰, respectively. The $\delta^{13}C$ values fall into the group for tea samples, suggesting a natural origin. In contrast, the $\delta^{13}C$ values of the other two tea drinks (−33.3 and −35.5‰) fall into the group for synthetic caffeine, indicating a synthetic origin. For the ready-to-drink products containing guaraná extract, the $\delta^{13}C$ values are between −26.3

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and −28.3‰ lying within the range for guaraná seeds (−25.3 to −28.7‰). No mislabeled guaraná drinks were found in this study. However, one maté drink was found to be probably mislabeled since the caffeine $\delta^{13}C$ value was −35.3‰. It is interesting to note that two different cola products show identical $\delta^{13}C$ values of −30.2‰, indicating a natural source of the kola nut extract. Among these 38 drinks, 6 tea drinks and 2 guaraná drinks were found to contain caffeine lower than 30 mg L^{-1}. The latter results are not included in Figure 5 because the concentration was below the detection limit of HT-RPLC/IRMS.

CONCLUSIONS

Compound-specific isotope analysis by HT-RPLC/IRMS has been applied to the measurement of caffeine in various drinks, allowing us to differentiate between natural and synthetic caffeine. Two separate ranges of $\delta^{13}C$ values for natural and synthetic caffeine have been identified based on the results in this work and in the literature: one from −25 to −32‰ and the other from −33 and −40‰. The method has been applied to control the stated natural caffeine sources in 38 drinks, out of which four products were found to be mislabeled due to added synthetic caffeine. The advantages of the presented method include simple sample preparation, short analysis time, long-term column stability, and high precision of $\delta^{13}C$ values. It has the potential to become a routine method for authenticity control of caffeine-containing drinks.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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