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Caffeine ID™

Trace determination of caffeine by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry

Submitter: Blue River

Submitter #'s: BR-1, BR-2, BR-3, BR-4

Source Molecular #'s: SM 0056, SM 0057, SM 0058, SM 0059

Samples Received: January 3rd, 2011

Date Reported: January 10th, 2011

SAMPLE

| SM # | Client # | Caffeine Concentration (ng/L) | Mass Spectrometry Results |
|---------|----------|-------------------------------|---------------------------|
| SM 0056 | BR-1 | 21.8 | Caffeine Detected |
| SM 0057 | BR-2 | < LOD* | Negative |
| SM 0058 | BR-3 | < LOD* | Negative |
| SM 0059 | BR-4 | 41.2 | Caffeine Detected |

* < LOD indicates a detected concentration below the 4 ng/L detection limit.

Laboratory Comments

Four 1-liter water samples were filtered and analyzed for the presence of unmetabolized caffeine. The analytical method used for these samples is able to detect the presence of unmetabolized caffeine in as low as 4 ng/L. Research has shown that caffeine can be used as a stable and good marker for human activities, and in particular as a proxy for human fecal contamination.

Samples BR-2 (Our Ref: SM 0057) and BR3 (Our Ref: SM 0058) were below the detection limit of 4 ng/L, therefore the samples were classified as negative for the presence of caffeine. Negative samples should be analyzed with other molecular and genetic methods described below in order to demonstrate conclusively that there is no presence of human forms of contamination in the water system.

Samples BR-1 (Our Ref: SM 0056) and BR4 (Our Ref: SM 0059) were positive for the presence of unmetabolized caffeine suggesting that human forms of contamination are present in these water samples. Nonetheless, as with the negative samples, additional tests should be conducted to strengthen the results. The Human Enterococcus ID™, Human Bacteroidetes ID™ and Human Fecal Virus ID™ services can be used to validate the presence of human contamination.

Method Explanation

All samples were filtered through a combusted 0.45-µm glass fiber filter (Whatman) before analysis. A one liter volume of filtered water sample was transferred to a two liter separatory funnel, and the appropriate amount of internal standard (atrazine⁵) was added. After raising the pH of the sample to 8-9 with a 0.1 M sodium hydroxide solution, the sample was extracted using liquid-liquid extraction with three portions of 50 ml methylene chloride. The organic extracts were concentrated to a volume of 2 ml using a water bath and a Snyder column and further evaporated to dryness using a gentle stream of purified nitrogen. After reconstituting the samples to 500 µl of 1:1 methanol/water, they were analyzed by LC/MS.¹

Quantitative determination of caffeine in the final extracts was carried out by HPLC-APCI-MS in positive mode under selected ion monitoring (SIM) using a Navigator aQa system (Finnigan). A Luna HPLC column (150 X 4.6 mm I.D.) packed with a bonded C18 phase (5 µm) was used for the chromatographic separation (Phenomenex). The mobile phase was programmed to run in a linear gradient from 30% methanol / 70% water to 100% methanol at a flow rate of 1 ml/min. The total run time required for the analysis was 10 min with caffeine and atrazine⁵ eluting at 3.97 and 8.83 min, respectively. Internal standard linear calibration plots were constructed using eight points at 2.5, 5, 10, 25, 50, 100, 200 and 400 µg/ml.

Theory Explanation

Caffeine, 1,3,7-trimethylxanthine is ranked number one drug worldwide and is usually employed as a stimulant commonly found in coffee, tea, cocoa, soft drinks and chocolate. It is also a component in hundreds of prescription and over-the-counter drugs, ranging from analgesics to cold medicines.³ The average human consumes considerable amounts of caffeine. From coffee alone, an average American consumes 131 mg of caffeine per day. However, caffeine is extensively metabolized by humans with only approximately 3% excreted unchanged in the urine. Nevertheless, there is far more caffeine introduced to the sewer system by disposal of unconsumed coffee, tea or soft drinks down the sink, and rinsing of coffee pots and cups.

Research has suggested that the presence of caffeine in the environment can serve as an indicator of the presence of human sewage. Levels of caffeine in domestic wastewater have been measured to be between 20 and 300 µg/liter.⁴ Levels in receiving waters can however be much lower due to significant dilution. Consequently, any analysis must be able to determine caffeine in the low part parts per trillion levels (4 ng/L LOD) in both freshwater and saltwater. The method described in the section above overcomes many of the previous limitations in detecting caffeine and is able to detect caffeine as low as 4ng/L.^{1, 2}

Caffeine is considered a good, stable, dissolved marker directly related to human activities with no potential biogenic sources because of its high solubility (13.5 g/l), low octanol/water partition coefficient (log Kow = 0.01) and negligible volatility.¹ This is of particular importance in environments where septic tanks contribute large amounts of the wastewater discharges in comparison with treated municipal wastewaters. As such, the detection of caffeine can demonstrate the presence of failing septic systems and other forms of human contamination.

¹ Gardinali PR, Zhao X. **Trace determination of caffeine in surface water samples by liquid chromatography -- atmospheric pressure chemical ionization--mass spectrometry (LC-APCI-MS).** Environ Int. 2002 Dec; 28(6):521-8.

² Bendriss E, Markoglou N, Wainer IW. **Liquid chromatographic method for the simultaneous determination of caffeine and fourteen caffeine metabolites in urine.** J Chromatogr, B 2000;746:331-8.

³ Seiler RL, Zaugg SD, Thomas JM, Howcroft DL. **Caffeine and pharmaceuticals as indicators of wastewater contamination in wells.** Ground Water 1999;37:405- 10.

⁴ Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. (2002) 68: 5796-5803.

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