Analysis of Caffeine by Isotopic Dilution Using Mass Spectroscopy

Adapted from Devon W. Hill, Brian 1. McSharry, and Larry S. Trzupek, J. Chem. Ed., 65, 907 (1988)

Background

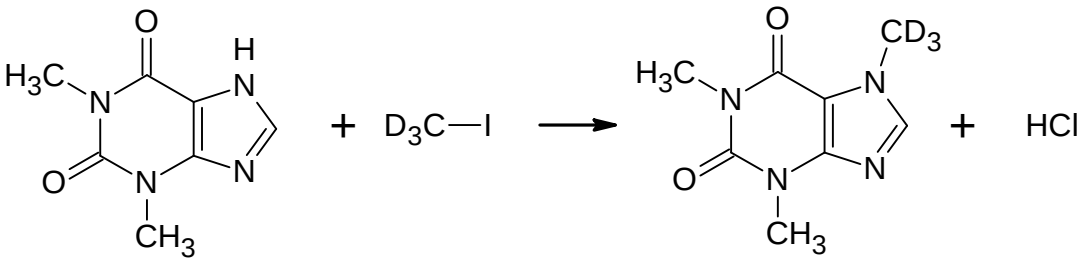
Direct Isotope Dilution Analysis – In this variation of isotope dilution analysis the amount/concentration of an analyte is determined by spiking the analyte containing matrix with a known quantity of an isotopically labeled analogue of the analyte or by spiking the analyte matrix with a known quantity of a closely related compound. The spike and the analyte, in general would be difficult to separate from the each other by conventional means. In the isotopic dilution method a signal associated with the analyte of interest is compared to that of the analogue/spike.

In this experiment caffeine is the analyte and tri-deuterated caffeine molecule would be the analogue mixed with it in the isotope dilution analysis protocol. GC-MS instrumentation in the "specific ion monitoring", or "SIM" mode is employed in the analytical procedure. In this configuration, the mass spectrometer samples the effluent GC stream only for ions of specifically designated masses or small ranges of designated masses. In the SIM mode a small number of distinct ions are monitored many times per second by the detector. Such cyclical and fast monitoring of ions in a small mass range or a single ion improves both the sensitivity and the analytical specificity of the technique.

Examination of caffeine and caffeine-d_3 show prominent peaks at 194 (197), 109 (112) and 82 (85) – deuterated compound peak in parenthesis. Analysis is based on the areas of the most prominent peak from each compound.

Procedure

*** Synthesis of caffeine-d_3**



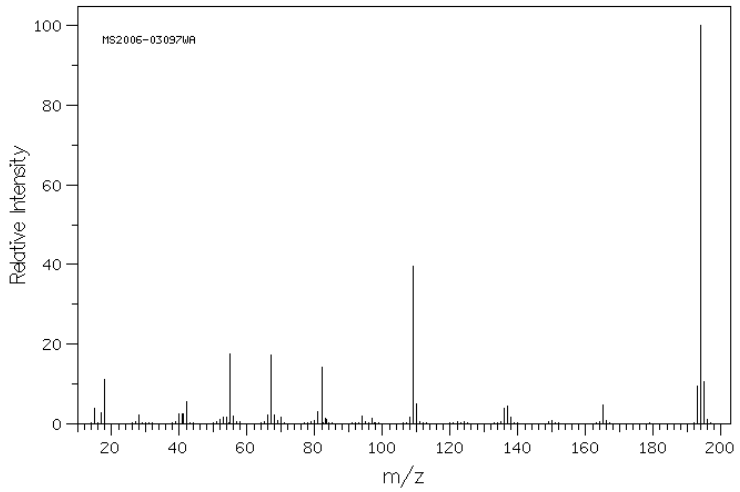
caffeine d_3

Place a solution of 2.00 g of theophylline (11.1 mmol) in 50 mL of dimethylformamide in a 125-mL Erlenmeyer flask equipped with a Teflon-coated magnetic stirring bar. To this stirred solution add 1.1 mL of 10 M aqueous sodium hydroxide (11 mmol). Stir for 1

min, and add 2.2 mL of iodomethane-d_3 (5.0 g, 34.5 mmol) in two portions over a period of 5min. Seal the flask with a septum stopper, and stir the mixture for 3 h, during which time a fine precipitate forms. Leave the mixture overnight in the freezer, and isolate the resulting precipitate by suction filtration. Break up the filter cake and wash with anhydrous ether to yield 7,7,7-caffeine-d_3 as a fine white solid.

Calibration: Determination of Response Factor

The first step in this procedure is to calibrate the spike against a known amount of the analyte. Calibration results in the response factor which relates the instrumental responses of the analyte and the spike. The MS of caffeine and caffeine-d_3 are very similar in appearance but for the three mass units difference on the m/z axis.



MS of caffeine (70eV)

http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng

15.0	3.8
17.0	2.6
18.0	11.2
28.0	2.1
40.0	2.4
41.0	2.5
42.0	5.4
52.0	1.1
53.0	1.6
54.0	1.7
55.0	17.5
56.0	2.0
66.0	2.2
67.0	17.3
68.0	2.1
70.0	1.6
81.0	3.1
82.0	***14.2***
83.0	1.4
83.5	1.1
94.0	1.9
97.0	1.4
108.0	1.7
109.0	***39.7***
110.0	4.9
136.0	3.8
137.0	4.4
138.0	1.7
165.0	4.8
193.0	9.3
194.0	***100.0***
195.0	10.6
196.0	1.0

The response factor R, for the system is defined from,

$$\text{area}\,@197 \;\alpha\; \text{mass caffeine-d}_3$$
$$\text{area}\,@194 \;\alpha\; \text{mass caffeine}$$

$$\frac{\text{area}\,@194}{\text{area}\,@197} = \text{R}\,\frac{\text{mass caffeine}}{\text{mass caffeine-d}_3}$$

Prepare four calibration standard solutions to determine R. Typically two stock solutions are made; 50mg of caffeine in 50.00mL methanol and 50.00 mg caffeine-d_3 in 50.00mL methanol. Make the four calibration standards by mixing 500uL of caffeine stock with 500uL, 375uL, 250uL and 125uL of caffeine-d_3 stock in four 1.00 mL volumetric flasks (or double the volumes if 2mL volumetric flasks are available). After dilution to the mark with methanol the mass ratios of (caffeine- d_3/caffeine) in the three solutions would be 1:1, 3:4, 1:2 and 1:4 respectively (table below).

All four standard solutions may be made directly in the GC-MS vials. Stopper them tightly immediately after transferring the components and mix.

If the masses are different from the ones suggested above perform the *appropriate accurate calculation* to find the actual mass ratios. Chromatograph the samples, GC-MS in SIM mode (please see below).

Plot $(\text{area @194} / \text{area @197})$ vs. $(\text{mass caffeine} / \text{mass caffeine-}d_3)$ for the four standards to estimate the value of R (slope) and the intercept.

Preparation of Laboratory sample

A caffeinated beverage with CO_2 expelled by boiling will be the sample. Dilute the unknown sample provided accurately 1 to 10. Mix 2.00mL of the diluted beverage with 100uL of the standard stock caffeine-d_3 (of concentration ~1.00 mg/mL of caffeine-d_3) in a clean test tube. Isolate the caffeines in this solution via solid phase extraction as described below.

Condition the SPE sorbent: activate the SPE cartridge stationary phase (Bakerbond – C18) by passing 6mL 50% methanol through the sorbent slowly; use a vacuum manifold. Make sure to keep the sorbent wet. *Turn off the vacuum as methanol level approaches the top of packing.*

Pass 3mL of water through the packing; keep the sorbent wet and turn off the vacuum as water level approaches the top of packing.

Introduce the solution prepared above onto the *preconditioned* SPE cartridge and apply a vacuum. Wash the cartridge with 2 mL of distilled water. Draw air through the SPE column for 3-5 minutes until the sorbent is dry.

Elute the adsorbed analytes (caffeines) from the SPE column with 2 mL of methanol: acetone: methylene chloride solvent mixture (5:1:15 by volume) into a clean test tube and transfer all of the solution eluted step into a vial. Dry off the vial by passing a stream of nitrogen through it carefully; re-dissolve the residue in 2.00 mL of methanol. This is the laboratory sample for GC-MS.

(GC) MS of solutions

Perform a GC-MS analysis of the laboratory sample in the SIM mode. The caffeine and the deuterated caffeine elute very closely and it is difficult to separate chromatographically. Every standard and the test trials are run in SIM at m/z 194 and 197 separately because the parent peaks are the base peaks as well.

Prepare the four standard solutions (all containing the deuterated caffeine) from the stock standards of caffeine and caffeine-d_3, methanol; and the test sample by mixing unknown solution with the deuterated caffeine stock solution as shown in the table below.

All four standard solutions may be made directly in the GC-MS vials. Stopper them tightly immediately after transferring the components and mix.

Concentrations: caffeine stock and caffeine-d_3 stock are around ~1mg/mL; calculate their actual concentrations before proceeding any further.

Standards	Caffeine-d_3 (uL)	Caffeine (uL)	Methanol (uL)	$\frac{\text{mass caffeine}}{\text{mass caffeine-}d_3}$	$\frac{\text{area @194}}{\text{area @197}}$
Std 1	500	500	-		
Std 2	375	500	125		
Std 3	250	500	250		
Std 4	125	500	375		

Test solutions	Caffeine-d_3 (uL)	Unknown (uL)	$\frac{\text{area @194}}{\text{area @197}}$	$\frac{\text{mass caffeine}}{\text{mass caffeine-}d_3}$
Trial 1	100	2000		
Trial 2	100	2000		
Trial 3	100	2000		

Calculate the caffeine content in the unknown sample in mg/L. and the standard error.

Save the caffeine-d_3 standard.