Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (9)

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Sport und Buch Strauß, Köln, 2001

A.T. CAWLEY, G.J. TROUT, R. KAZLAUSKAS:
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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (9). Sport und Buch Strauß, Köln, (2001) 229-232

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Quantitation of Urinary Caffeine by GC/MS using 13C Caffeine as Internal Standard

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BACKGROUND

This paper describes a method for the quantitation of caffeine in the urine of athletes by Isotope Dilution Mass Spectrometry (IDMS) using ^{13}C labelled caffeine. We have found that the accuracy and precision at the target concentration of 12µg/mL is as good as the HPLC method which is recommended in the IOC Code. The procedure is simple and the extraction method follows the routine screen I procedure used by IOC accredited laboratories. The Screen I procedure, specifically that using the 6890 HP GC/NPD, allows for a very large range of concentrations to be measured due to the good dynamic range of this instrument. Thus caffeine at levels above 10µg/mL allows this substance to be quantified at the same time as screening for small amounts (<0.5µg/mL) of other drugs.

The current accepted IOC method of caffeine quantitation requires analysis by High Performance Liquid Chromatography (HPLC) as well as confirmation by GCMS. Thus several different processes have to be undertaken before the result can be reported. HPLC quantification does not provide quantitation and identification from the same extract. A GCMS method would perform these tasks simultaneously and save considerable effort.

METHOD

Trimethyl ^{13}C caffeine is added at 10µg/mL and in addition to diphenylamine (DPA) at 12µg/mL to the urine. Screen I extraction (see below) procedure is undertaken with analysis using MSD in SIM mode using ions for caffeine (m/z=194) and ^{13}C caffeine (m/z=197). Full scan spectra are also obtained for identification compared with spectra of standards run at the same time.

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Reagents and Chemicals

- Caffeine (Anhydrous, 99.9%) was purchased from Boehringer Ingelheim (Germany), caffeine (trimethyl-$^{13}C_3$, CLM-514, Anhydrous, ^{13}C 99%; +98%) was purchased from Cambridge Isotope Laboratories (Andover, USA).
- A caffeine stock standard solution of 1 mg/mL was prepared by dissolving 10mg anhydrous caffeine in 10mL methanol in a volumetric flask. A 100 mg/mL working solution was then obtained by a 1 in 10 dilution of this stock solution in methanol.
- A ^{13}C caffeine standard (**CS**) of 1.06 mg/mL was prepared by dissolving 10.6mg ^{13}C caffeine in 10mL of methanol using a volumetric flask.
- A DPA standard (**DS**) of 1 mg/mL was prepared by dissolving 10mg Diphenylamine (DPA) in 10mL of methanol using a volumetric flask.

Calibration Spikes

A five-point calibration curve consisting of 0,5,10,15 and 20µg/mL caffeine in blank urine was prepared with each validation concentration.

Sample preparation

To 5mL urine, 20µl **CS** and 20µl **DS** were added as internal standards. t-Butylmethyl ether (2.5mL) and potassium hydroxide (6M, 0.5mL) were then added followed by anhydrous sodium sulfate (2g). The mixture was shaken on a rotary shaker for 20min before being centrifuged for 10min. The organic layer was then transferred to a GC vial for analysis.

Analysis by GC-MS

- All analyses were carried out on a Hewlett-Packard 5890 gas chromatograph coupled to a H-P 5970 mass selective detector using a 30m X 0.25mm SGE BPX-50 (50%-phenyl)-methyl silicone (film thickness, 0.25um) capillary GC column; split mode (1:10); injection temperature of 250^0C; initial temperature 70^0C ramped at 12^0C/min to 190^0C, then 30^0C/min to 310^0C; transfer line 300^0C; Injection volumes 2µl.
- SIM mode, with a solvent delay of 2.5min; Ions at m/z 109.05(30ms), 111.05(10ms), 166.1(30ms), 169.1(30ms), 168.1(10ms), 194.1(30ms), 195.1(10ms), 196.1(10ms) and 197.1(30ms) were monitored.
- Caffeine and ^{13}C-caffeine RT 12.2min. DPA eluted at 9.2min.
- Calibration curve $\mathbf{y = 0.2186x + 0.062}$ $R^2 = 0.9998$ for ^{13}C-caffeine as INSTD based on Area ratio m/z 194/197

METHOD VALIDATION

The proposed method was validated by the extraction and analysis of seven prepared samples of four concentrations; 0µg/mL, 1µg/mL, 10µg/mL and 12µg/mL. A set of calibration spikes were prepared, extracted and analysed with each of the four sample concentrations.

Reproducibility

	C-13 as INSTD	DPA as INSTD
Mean (1µg/mL)	1.14	0.50
S.D	0.048	0.102
% C.V	4.20	20
Mean (10µg/mL)	9.68	9.60
S.D	0.236	0.466
% C.V	2.44	4.85
Mean (12µg/mL)	12.00	12.802
S.D	0.205	0.979
% C.V	1.71	7.649

CONCLUSION

- The GCMS method gives acceptable precision and accuracy;
- The ^{13}C caffeine gives better results than DPA which is a common internal standard for screen 1.
- The IOC Code should not define the quantitation method for caffeine as only by HPLC;
- Both HPLC and GCMS may be used for caffeine quantitation

See also D3-caffeine: Quantitative Analysis by Isotopic Dilution Using Mass Spectrometry, The Determination of Caffeine by GCMS; Hill, D.;McSharry, B.; Trzupek, L. *J. Chem. Educ.* **1988,** 65, 907-910.