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Gas Chromatography Analytical Methods for Teaching Laboratories

Quantitative analysis of caffeine in urine by GCMS

Introduction

The screening of human urine samples for drugs of abuse is a method routinely used by forensic laboratories. This application aims to simulate this using a stimulant drug that is legal and easily available, caffeine.

This application utilizes a GCMS with a standard inlet to detect the presence of caffeine in a complex matrix, such as urine or artificial serum. The target analyte is identified in a complex matrix using a mass spectral library, such as NIST. A caffeine standard will be serially diluted and used to prepare a calibration curve from which the concentration of caffeine in the test sample can be determined.

Reagents

Dichloromethane – analytical grade

Methanol - analytical grade

9:1 solution of dichloromethane:methanol (vol)

Saturated solution of sodium chloride

Caffeine analytical standard – 1.0 mg/mL in methanol, C6035 Supelco or suitable equivalent.

Caffeine powder – ReagentPlus, C0750 Supelco or suitable equivalent

Sigmatrix urine diluent – SAC0074-1L Sigma-Aldrich or suitable equivalent.

Safety

Lab coats, safety glasses and gloves should be worn when handling reagents.

Follow all appropriate safe operating procedures and beware of any specific actions raised in the associated risk assessments.

Apparatus

- Gas chromatograph fitted with a split/splitless injection port and mass spectral detector (MS).
- NIST Mass Spectral Library or suitable equivalent.
- Autoinjector and autosampler capable of handling liquid samples in 1.5 mL vials
- SH-I-5MS column, 30 m x 0.25 mm I.D. x 0.25 µm, p/n 221-75940-30 (or suitable equivalent).
- Centrifuge
- 50 mL centrifuge tubes
- 1.5 mL autosampler vials and caps.
- Appropriate laboratory glassware for the preparation of the standard and samples.

Instrument Conditions

GCMS Parameters	
Injections Temperature	280°C
Injection Mode	Split
Split Ratio	1:10
Purge flow	6 mL/min
Column	SH-I-5MS, 30 m x 0.25 mm x 0.25 µm
Oven Program	100°C initial temperature, hold 1 minute 40°C/min to 210°C and hold for 0 minutes 5°C/min to 225°C and hold for 0 minutes 40°C/min to 300°C and hold for 2 minutes
Run Time	10.63 minutes
Carrier Gas	Helium, flow 2.0 mL/min
Injection Volume	1.0 µL
Transferline Temperature	280°C
Source Temperature	230°C
MS Scan range	50 to 350 m/z

Preparation of individual solvent standards

Prepare an intermediate caffeine solution by diluting the stock standard 1 in 10 in dichloromethane.

Use the table below to prepare calibration standards by diluting the intermediate solution in dichloromethane in a 1.5 mL vial.

Standard concentration mg/L (ppm)	Volume of intermediate (µL)	Volume of dichloromethane (µL)
Blank	0	1000
0.1	1	999
1.0	10	990
5.0	50	950
10.0	100	900
50.0	500	500

Preparation of test sample

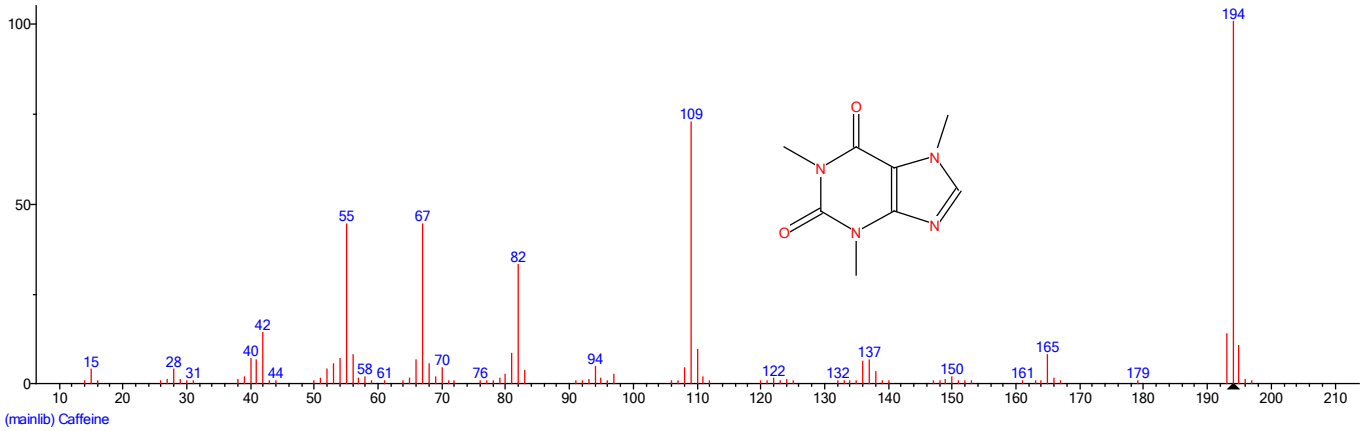
Prepare a test sample between 0.5 and 2 ppm (a typical range found in caffeine drinkers) by dissolving the appropriate amount of caffeine in the Sigmatrix urine diluent.

Extraction of the test sample

- Add 5 mL of the 9:1 dichloromethane:methanol solution to 25 mL of the test solution and shake.
- Add 1 mL of saturated sodium chloride solution and centrifuge at 2000 rpm for 5 minutes. Add a further 1 mL of saturated sodium chloride solution if the emulsion does not separate.
- Transfer 1 mL of the bottom organic layer to a 1.5 ml vial for analysis.

Interpretation of results

Using the GCMS in scan mode will give a spectrum for each compound that has been extracted from the sample and detected by this technique. The similarity search function in the data processing software can then be used to compare these spectra to the NIST library and provide a tentative identification. Caffeine will elute at approximately 5.5 minutes.



The calibration standards can be analyzed to create a six-point linear calibration curve. This can then be applied to determine the concentration of caffeine in the test sample. Note: that the test sample undergoes a x5 concentration during extraction, so an appropriate factor will need to be applied to the result.

Key learning outcomes

- How compounds in a complex matrix can be identified by comparing their mass spectrum to those held in a database such as the NIST mass spectral library.
- Accurate preparation of stock solutions and standards.
- How to create a calibration curve using analytical standards and using it to determine the concentration of the target analyte in a test sample.

Notes

The instrument conditions are based on published methodologies. If an alternative column or carrier gas is used, these conditions will need to be adapted appropriately.