

Determination of Drugs in Blood Plasma by GC/MS

References

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Goal

To determine the identity and quantity of any illicit drugs present in a blood plasma sample or urine sample.

Suggested Method of Analysis

Solid phase extraction to isolate the drug; gas chromatography – mass spectrometry for identification and quantitation

Preparation of standards, samples, and reagents

Prepare a working standard solution containing about 10 $\mu\text{g}/\text{mL}$ of the drug in methanol. Use a syringe or pipet to place a few μL of drug stock solution in a 5-mL volumetric flask. Dilute with methanol to the mark, mix the solution, and store some of the standard in a glass chromatography vial and refrigerate it. The working standard solution may only be good several days. Use this solution to confirm the chromatographic determination and mass spectrometry of the drug.

Prepare an aqueous standard solution containing about 0.50 $\mu\text{g}/\text{mL}$ of an illicit drug. Use a syringe or pipet to place a few μL of drug stock solution in a 50-mL volumetric flask. Dilute with water to the mark, mix the solution, and store the sample in a glass vial. Refrigerate. The standard solution may only be good several days. Use this solution to check the collection efficiency of the solid phase extraction process.

Solid phase extraction of a drug from a blood plasma or urine sample

Condition a mixed-mode (C8 and strong cation exchanger) cartridge (3-mL volume; 100 mg resin), load the sample, wash the cartridge, and elute the drug in the following manner:

	flow rate:
<i>condition</i>	
3.0 mL methanol	5 mL/min
3.0 mL 0.050 M, pH 6.0 phosphate buffer	5 mL/min
<i>load</i>	
5.0 mL sample plus 3 mL pH 6.0 buffer	2 mL/min
<i>wash</i>	
3.0 mL water	2 mL/min
3.0 mL 0.1 M HCl	2 mL/min
3.0 mL methanol	2 mL/min
<i>elute</i>	
5.0 mL 80:18:2 ethyl acetate: methanol : NH ₄ OH	2 mL/min

Set the flow rate by adjusting how much vacuum is applied. Do not let the resin dry out at any time during the procedure. Elute the drug with 5 mL of mixed solvent, at a flow rate of 2 mL/min, into a disposable test tube. Aspirate about 1 minute at higher vacuum to collect all of the eluate.

Reduce the volume of the eluate to just about zero by solvent evaporation (warm the solution to 50 °C while flowing nitrogen gas over top of it). Perform the evaporation, which takes about 30 min, in a fume hood. Add 0.50 mL of methanol to redissolve the drug, and then transfer the processed sample to a 2-mL chromatography vial. Store the solution in the refrigerator. Perform the GC-MS analysis as soon as possible.

Gas chromatography – mass spectrometry

The oven temperature program for the gas chromatograph is:

Initial value: 175 °C	Initial time: 2.0 min
Rate: 15 °C /min	
Final value: 225 °C	Final time: 5.0 min

Use a split injection of 5 µL with a 20:1 split ratio. The flow rate of helium is 1 mL/min. The mass spectrometer is set to scan from 50 to 350 m/z with a start time of 2.0 min. Record the total ion chromatogram, the peak areas, and the mass spectrum of the drug(s).

Compare the mass spectrum generated by the drug in the simulated plasma sample to known spectra of the possible drugs (prepare a list of possibilities for the students). Find known mass spectra in an electronic library that is part of the mass spectrometer software or in printed compendia from the library*. In the former case, one can also perform a library search and obtain the best matches. Once the drug is identified, the peak areas of the standard and sample can be compared and calculations made to estimate the concentration of the drug in the sample.

*Some mass spectra of drugs can be found in *The Wiley/NBS Registry of Mass Spectral Data*. For example:

- heroin (vol. 4, p. 4165, fig. 11)
- phenobarbital (vol. 2, p. 1645, col. 1, fig. 3)
- morphine (vol. 3, p. 2484, col. 2, fig. 11)
- cocaine (vol. 3, p. 2901, fig. 6).