Comparison of HPLC and GC–MS for Measurement of Cocaine and Metabolites in Human Urine

Diane L. Phillips and **Ian R. Tebbett***
Department of Pharmaceutics, Box 100494, University of Florida, Gainesville, Florida 32610

Roger L. Bertholf
Department of Pathology, University Medical Center, 655 W. 8th Street, Jacksonville, Florida 32209

Abstract

High-performance liquid chromatography (HPLC) was compared with gas chromatography–mass spectrometry (GC–MS) for quantitation of cocaine, benzoylecgonine, norcocaine, and cocaethylene in urine. Calibration standards were prepared in human urine, and bupivacaine was added as the internal standard for quantitation. After solid-phase extraction, the reconstituted samples were divided into aliquots for analysis by HPLC and GC–MS. The analytical performance of the two methods were compared with regard to sensitivity, precision, and dynamic range. Results of GC–MS and HPLC analyses of nine urine specimens previously confirmed as positive for benzoylecgonine were compared. Analytical results by HPLC were comparable to GC–MS. Therefore, for many laboratories, HPLC is a useful alternative to GC–MS for measuring cocaine and metabolites in urine.

Introduction

Analytical techniques used for measuring cocaine and metabolites include gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC). GC–MS provides the highest degree of specificity and is the accepted reference method of analysis for cocaine, benzoylecgonine, and ecgonine methyl ester (1–7). However, the application of GC–MS is limited to volatile analytes, and some amount of pre-analytical derivatization is frequently required. The use of HPLC is becoming more common as the advent of diode-array and multiwavelength detectors has improved the selectivity of the method by giving ultraviolet (UV) absorption profiles and derivative spectral data for each peak in the chromatogram. Very sensitive HPLC methods have been developed for detection and quantitation of cocaine and its metabolites in a variety of biological matrices (8–13). The main drawbacks of this HPLC application are the lack of a useful UV absorption band for ecgonine methyl ester and greater susceptibility to matrix interferences in comparison with GC–MS.

*Author to whom correspondence should be addressed.

With the exception of a study to certify the National Institute of Standards and Technology Standard Reference Material for benzoylecgonine and cocaine (14), no data have been published that demonstrate how closely HPLC methods compare with GC–MS for quantitative analysis of cocaine and metabolites. The purpose of this work was to determine whether HPLC analysis of urine samples containing cocaine and metabolites produced comparable precision, sensitivity, and reproducibility to GC–MS analysis of the same samples.

Materials and Methods

Materials

Methanol, acetonitrile, and monobasic potassium phosphate (all HPLC grade) were purchased from Fisher (Fair Lawn, NJ). Butylamine, acetic acid, and ammonium hydroxide were reagent grade (Fisher). Cocaine hydrochloride, benzoylecgonine, and bupivacaine were purchased from Sigma Chemical (St. Louis, MO). Cocaethylene and norcocaine were provided by the National Institute on Drug Abuse (NIDA, Rockville, MD). Pentafluoropropionic anhydride, pentafluoropropanol, and dimethylformamide were purchased from Aldrich (Milwaukee, WI).

GC–MS instrumentation

A Finnigan MAT (San Jose, CA) Incos 50 quadrupole MS, Hewlett-Packard (Palo Alto, CA) model 7673A autoinjector, and a Hewlett-Packard model 5890 GC were used for the quantitative analysis of cocaine (CO), benzoylecgonine (BZE), norcocaine (NC), bupivacaine (BUP), and cocaethylene (CE). The compounds were separated on a DB-1 fused-silica capillary column coated with methyl silicone bonded phase (30 m × 0.32-mm i.d., 0.25-µm film thickness; J&W Scientific, Folsom, CA). The injector was operated in the splitless mode at 280°C, and helium was used as carrier gas at a column head pressure of approximately 6 psi and a flow rate of 1 mL/min. Specimens (2 µL) were injected at 50°C; the split valve was opened after 1 min; and the temperature was increased to 280°C at a rate of 33°C/min. The ion source was operated at 180°C with an

accelerating voltage of 70eV, 750 µA filament current, and 1 kV electron multiplier voltage (the conversion dynodes operated at 5 kV). Ion current was acquired at the following masses: CO: 82, 182, 303; BZE: 300, 316, 421; NC: 194, 313, 435; CE: 196, 272, 317; BUP: 140. The total scan time was 0.439 s.

HPLC instrumentation

The HPLC system used a Waters (Milford, MA) model 501 pump to deliver mobile phase at 1.5 mL/min to a Lichrosorb RP-18 10 µm (25 cm × 4.6-mm i.d.) analytical column (Alltech, Deerfield, IL). A C_{18} Novapak Guard pak precolumn (Waters) was used to protect the analytical column. The mobile phase consisted of 0.025M monobasic potassium phosphate buffer–acetonitrile–butylamine (81:18:1, v/v/v) adjusted to pH 3.0 with *o*-phosphoric acid. The sample was injected using a Waters WISP 710B autosampler with a capacity for 48 samples. The volume of the sample injected was 50 µL. The detector was a Waters model 486 tunable absorbance detector and the eluent was monitored at 230nm. Instrument control, data acquisition, processing, and reporting were handled using a NEC (Boxboro, MA) Powermate 386 computer and Waters Millennium 2010 (version 2.0) Chromatography Manager software. In addition, this system also included Waters Millennium System Suitability software which provided for trend plotting, quality control, and method validation following GMP/GLP regulatory protocols.

Experimental

Quantitation of urinary cocaine and metabolite concentrations was based on a six-point calibration curve. Calibration standards were prepared by spiking drug-free urine to concentrations of 50, 100, 200, 400, 750, and 1500 ng/mL. A standard stock solution was prepared by adding 10 µg/mL of each of cocaine, benzoylecgonine, norcocaine, and cocaethylene to drug-free urine obtained from a volunteer. Aliquots of this solution were used to spike drug-free urine in the preparation of calibration standards. Bupivacaine (0.5 µg/mL) was added to individual standards and samples prior to solid-phase extraction (SPE). Drugs were extracted from the urine onto SCX solid-phase ion exchange columns (1-mL size, Varian, Harbor City, CA) after preconditioning with methanol (2 mL), water (1 mL), and 0.25M phosphate buffer (pH 3, 1 mL). Urine specimens were diluted 1:1 with 0.25M phosphate buffer before addition of internal standard. The sample was applied to the extraction column and aspirated slowly under slight negative pressure. The column was dried under vacuum for 30 s and then washed with phosphate buffer (1 mL), 0.1M acetic acid (0.5 mL), and methanol (1 mL). After drying the column again for 30 s, the drugs were eluted with 3% ammonium hydroxide in methanol (1.5 mL). The SPE eluant was evaporated to dryness under nitrogen, reconstituted in 100 µL methanol, and half of the volume was transferred to a silanized Reacti-vial™ (Varian) to be derivatized prior to GC–MS analysis. Both aliquots were again evaporated to dryness at 60–70°C under a gentle ni-

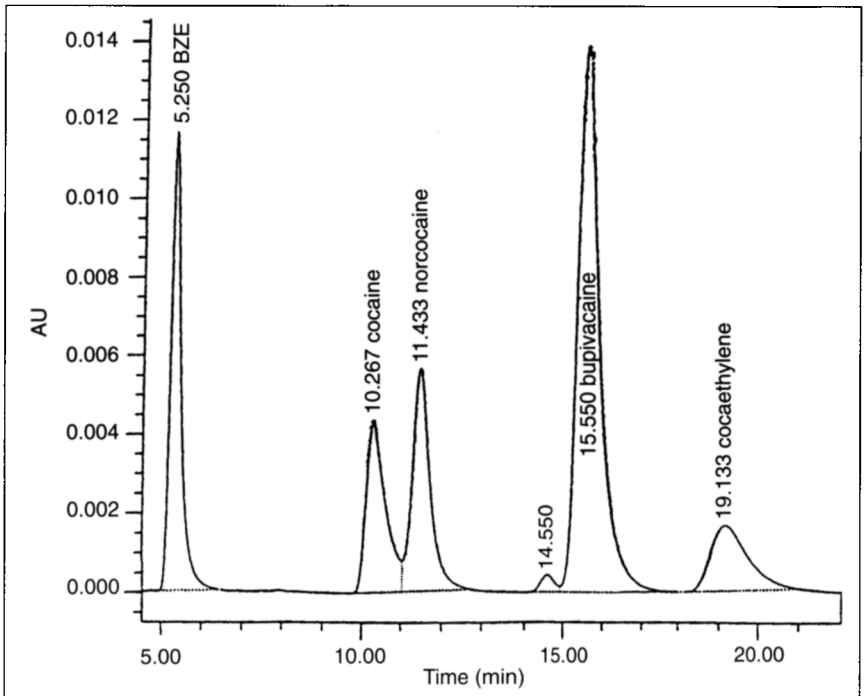

Figure 1. Representative HPLC chromatogram showing the separation of benzoylecgonine (BZE), cocaine, norcocaine, bupivacaine, and cocaethylene. The peak eluting at 14.55 min is due to an endogenous compound in the urine extract.

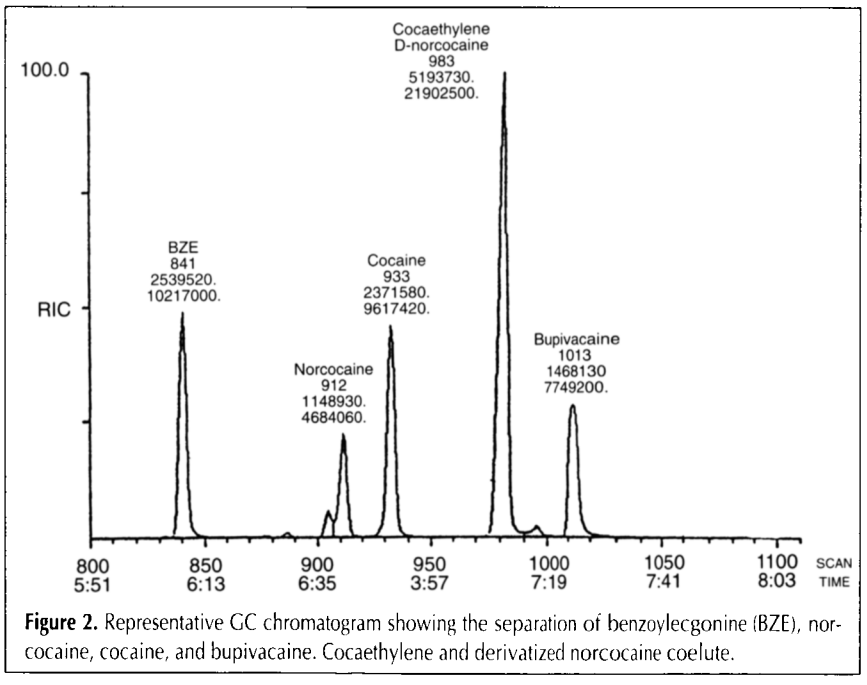

Figure 2. Representative GC chromatogram showing the separation of benzoylecgonine (BZE), norcocaine, cocaine, and bupivacaine. Cocaethylene and derivatized norcocaine coelute.

trogen stream. The aliquot designated for HPLC was reconstituted in 100 µL mobile phase prior to analysis.

Each analyte was calibrated three times over a 2-week period. Nine human urine specimens identified as cocaine positive by a certified drug testing laboratory were also analyzed by the procedure described previously. Finally, interday variability was determined by replicate analyses of seven standard samples containing all analytes, which were divided and analyzed according to the previously described procedure. The limit of detection (LOD) was determined by analysis of progressively lower concentrations of cocaine and metabolites until the analyte signal was no longer reproducibly distinguishable from noise. The limit of quantitation was defined as five times the LOD.

Derivatization

The following method was used for the derivatization of benzoylecgonine and norcocaine for GC–MS analysis. Pentafluoropropionic anhydride (50 µL) and pentafluoropropanol (25 µL) were added to the SPE residue. The vial was tightly capped and vortex mixed, and the derivatization reaction was allowed to proceed for 20 min at 78°C, after which time the reagent was evaporated under a nitrogen stream. The residue was reconstituted in 50 µL dimethylformamide prior to GC–MS analysis.

Statistical analysis

Slopes, intercepts, and correlation coefficients were determined for each calibration curve by linear regression. Precision for each analyte was evaluated by a paired Student's *t*-test on the interday variabilities of the methods by averaging the coefficients of variation of all the standard calibrators. The analytical sensitivities of the methods were compared by paired *t*-tests on the mean slope determinations for each analyte.

Results

Sensitivity and specificity are the customary measures of the detection limit and the susceptibility to endogenous interferences, respectively, of an analytical method. There was no evidence of chromatographic or spectral interferences in either the HPLC or the GC–MS methods used in this study. GC–MS is widely regarded as the most specific analytical method available for drug detection and quantitation, although there were no falsely positive or negative results by either method in this limited comparison. Figures 1 and 2 present representative HPLC and GC chromatograms, respectively.

Calibration results are presented in Table I and include the means of slopes, *y*-intercepts, and correlation coefficients for the three separate calibrations by each method. A comparison of the precision of GC–MS versus HPLC for each analyte is shown in Table II. HPLC showed greater precision, as shown by a significantly lower coefficient of variation, for the determination of cocaine, whereas GC–MS showed greater precision in the analysis for benzoylecgonine, norcocaine, and cocaethylene. The sensitivities of GC–MS and HPLC for the analysis of BZE and CE were not statistically different ($p < 0.05$). GC–MS demonstrated greater sensitivity for CO, and HPLC was more sensitive for NC ($p < 0.05$). Overall, GC–MS demonstrated better precision than HPLC, but the methods had generally equivalent sensitivities.

Quantitative results for benzoylecgonine in human urine samples previously identified as positive by a certified drug testing laboratory were in close agreement by both methods ($r = 0.9992$).

Discussion

Results of this comparison of HPLC analysis for cocaine and metabolites with GC–MS indicate that HPLC is a legitimate alternative method of analysis for certain analytes. In situations where structural identity is required for legal purposes, or for qualitative analysis of unknowns, mass spectrometry is clearly superior to UV spectroscopic methods of detection. However, in quantitative applications where the need for spectral data on individual analytes is less intense, HPLC with photodiode array or multiwavelength detection can provide comparable sensitivity and precision with limited specificity via UV and derivative spectra. An advantage of the HPLC method described here is that less sample pretreatment is required because both BZE and NC must be derivatized prior to analysis by GC–MS.

Although HPLC was more sensitive for cocaine, GC–MS had

Table I. Calibration Curve Comparisons for GC–MS and HPLC*

	Slope		Intercept (ng/mL)		$r^{\dagger}$	
Compound	Mean	SD	Mean	SD	Mean	SD
HPLC						
BZE‡	6.37	0.22	−12	15	0.9972	0.0019
Cocaine	3.90	0.15	4	18	0.9972	0.0034
NC	5.31	0.21	−41	8	0.9970	0.0074
CE	9.86	0.295	3	4	0.9972	0.0035
GC–MS						
D-BZE	6.63	1.30	27	70	0.9954	0.0111
Cocaine	6.07	0.85	25	62	0.9896	0.0168
D-NC	1.48	1.59	−34	86	0.9980	0.1009
CE	8.37	0.49	51	76	0.9942	0.0068

* $N = 3$ calibrations. Curves were constructed by plotting concentration (*x*-axis) against the ratio of the response of the analyte to that of the internal standard (*y*-axis).
† r = correlation coefficient.
‡ Abbreviations: BZE = benzoylecgonine; NC = norcocaine; CE = cocaethylene; D-BZE = derivatized benzoylecgonine; D-NC = derivatized norcocaine.

Table II. Comparison of Method Precision

Analytical method	Interday variability (CV)			
	Benzoylecgonine	Cocaine	Norcocaine	Cocaethylene
HPLC	4.98	6.99*	3.79	7.18
GC–MS	1.98*	8.28	3.00*	5.99*

* $p < 0.05$ using paired *t*-test.

greater analytical sensitivity for norcocaine, and the two methods were comparable for benzoylecgonine and cocaethylene. For the HPLC method, the limits of detection were 1 ng/mL for benzoylecgonine, cocaine, and norcocaine and 2 ng/mL for cocaethylene. The LODs for GC–MS were 1 ng/mL for benzoylecgonine and cocaethylene, 2 ng/mL for cocaine, and 10 ng/mL for norcocaine. GC–MS demonstrated the lowest interday variability (1.98–8.28%) for all compounds except cocaine (7.0% for HPLC versus 8.3% for GC–MS). With the HPLC method, a linear response was observed for all compounds over the range of 50–1500 ng/mL. A linear response was observed with the GC–MS method for benzoylecgonine, cocaine, and cocaethylene over the range of 50–1500 ng/mL. However, the GC–MS method for norcocaine was problematic; the compound did not undergo reproducible derivatization and required daily calibration for accurate quantitation. An alternative derivatization procedure has been reported for norcocaine using hexafluoroisopropanol instead of pentafluoropropanol, which may obviate this problem (2). Excellent linear correlation was observed between HPLC and GC–MS for quantitative analysis of benzoylecgonine in human urine specimens (r = 0.9992).

Conclusion

In conclusion, we have shown that HPLC offers reproducibility and sensitivity comparable with GC–MS for the analysis of urine for cocaine and metabolites. HPLC does not have the specificity of GC–MS, thereby precluding its use in legal cases. However, the lack of a derivatization step and overall lower costs associated with liquid chromatography make it an attractive alternative to GC–MS for the determination of cocaine and metabolites in cases where structural confirmation is not required.

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