*Journal of Chromatography*, 422 (1987) 274–280
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3858

## Note

# Pharmacokinetics of intravenous caffeine: comparison of high-performance liquid chromatographic and gas chromatographic methods

JOHN S. KENNEDY, BARBARA W. LEDUC, JOSEPH M. SCAVONE, JEROLD S. HARMATZ, RICHARD I. SHADER and DAVID J. GREENBLATT*

*Division of Clinical Pharmacology, Departments of Psychiatry and Medicine, Tufts University School of Medicine and New England Medical Center, 171 Harrison Avenue, Boston, MA 02111 (U.S.A.)*

(First received March 11th, 1987; revised manuscript received July 17th, 1987)

Because caffeine is a ubiquitously available and extensively used psychotropic drug, understanding of its kinetic and dynamic properties is of clinical and scientific importance. To this end, sensitive and reliable methods are needed for quantitation of caffeine and metabolites in biologic fluids. A number of high-performance liquid chromatographic (HPLC) [1–5] and gas chromatographic (GC) [6–13] methods are available. Reported GC methods have limitations including use of clinically available drugs (phenacetin or phenobarbital) as internal standard (or no internal standard at all), the need for derivatization of samples prior to chromatography, non-linear detector response versus concentration relationships, or inadequate sensitivity [6–13].

The present report describes an automated GC procedure with nitrogen–phosphorus detection that can be used to quantitate underivatized caffeine in human plasma with high sensitivity. Applicability of the method to single-dose pharmacokinetic studies of caffeine in humans is demonstrated. Also demonstrated is a high degree of inter-method agreement between the GC procedure and an HPLC technique.

## EXPERIMENTAL

### *Reagents, glassware and standards*

Anhydrous caffeine, paraxanthine and the GC internal standard 7-(2-chloro-

0378-4347/87/$03.50

ethyl)theophylline were obtained from Aldrich (Milwaukee, WI, U.S.A.). Theobromine and the HPLC internal standard β-hydroxyethyltheophylline were obtained from Sigma (St. Louis, MO, U.S.A.). Theophylline was obtained from Chemlog (South Plainfield, NJ, U.S.A.). Other reagents were obtained from standard commercial sources.

Caffeine–sodium benzoate injection (USP) was obtained from Pharma-Serve (Queens Village, NY, U.S.A.).

A stock solution of caffeine (100 μg/ml), theophylline (50 μg/ml), paraxanthine (50 μg/ml) and theobromine (50 μg/ml) was prepared by weighing appropriate amounts of each standard individually, then dissolving them together in 100 ml of methanol. A working solution of the mixed standards was prepared by 1:10 dilution with methanol. The two internal standards were separately weighed and dissolved in methanol at concentrations of 40 μg/ml. All solutions were stored at 4°C in glass-stoppered bottles and were stable for at least three months.

### *High-performance liquid chromatography*

The HPLC instrumentation has been described previously [14–18]. The column (Supelco, Bellefonte, PA, U.S.A.) was stainless steel, reversed-phase, 15 cm × 4.6 mm I.D., packed with LC-18 DB (particle size 5 μm). The mobile phase consisted of 1.75 m*M* orthophosphoric acid–acetonitrile–tetrahydrofuran (97:02:01); organic and aqueous components were separately filtered and degassed prior to use. Operating conditions were: detector, 273 nm and 0.02 a.u.f.s.; mobile phase flow-rate, 1.2 ml/min; chart-speed, 0.30 cm/min. Injection volume was set at 15 μl.

### *Gas chromatography with nitrogen–phosphorus detection*

A Hewlett-Packard 5840A gas chromatograph equipped with a nitrogen–phosphorus detector, data module and an autoinjector set to deliver 6 μl per injection were used. The column was coiled glass (3 m × 2 mm) packed with 3% SP-2250 on 80–100 mesh Supelcoport (Packing 1-1767, Supelco). The carrier gas was ultra high pure helium at a flow-rate of 30 ml/min. The detector purge was ultra high pure hydrogen at 3 ml/min mixed with dry air at 50 ml/min. The operating conditions were: injection port temperature, 310°C; column temperature, 230°C; detector temperature, 300°C; chart-speed, 0.50 cm/min. Before being connected to the detector, the column was conditioned at 290°C for 48 h with a carrier flow-rate of 10–15 ml/min. The column was primed with 2 μg of purified soy phosphatides (Asolectin) in benzene (1 mg/ml) at the beginning of each working day.

### *Sample preparation and extraction*

For standard curve preparation, appropriate amounts of the mixed standards and 50 μl of each of the two internal standard solutions (containing 2 μg) were added to 13-ml screw-top tubes and evaporated to dryness under mild vacuum and a temperature below 40°C. For actual biological samples, only internal standards were added. Drug-free control plasma (0.5 ml) was added to calibration tubes, and 0.5 ml of "unknown" plasma added to other tubes. Each sample was analyzed in duplicate.

Ethyl acetate–isoamyl alcohol (98:2) (4 ml) and 200 $\mu$l of 0.1 $M$ hydrochloric acid were added to each sample, after which they were agitated in the upright position on a vortex mixer for 60–90 s. This solution was then centrifuged at room temperature for 10 min at 400 $g$. A 3-ml volume of the upper organic layer was transferred by pipette to conical centrifuge tubes for subsequent HPLC analysis. The remaining 1 ml was transferred to the GC autoinject vials. In both cases, the organic phase was evaporated to dryness. The residue for HPLC analysis was reconstituted in the conical tubes with 300 $\mu$l of mobile phase and transferred by pipette to the HPLC microvial insert. The dry residue for GC analysis was reconstituted in the autoinject vial with 200 $\mu$l of toluene–isoamyl alcohol–asolectin solution (83:14:3) and capped. Samples were stable for at least thirty days when stored frozen.

### *Human pharmacokinetic studies*

Eleven healthy volunteers, aged 19–42 years, participated after giving written informed consent. Subjects agreed to abstain from the use of coffee, tea, cola drinks and chocolate for three days prior to and during the study, and were taking no other medications. On the day of the study, they were admitted to the study unit where they received 300 mg of caffeine benzoate (equivalent to 150 mg of anhydrous caffeine), diluted in 50 ml of physiologic saline, by infusion into an antecubital vein over 15 min using a constant-rate infusion pump. Venous blood samples were collected from a separate site into heparinized tubes prior to injection and at 5 min and 0.25, 0.50, 1, 2, 3, 4, 6, 8, 10, 12, 15, 18 and 24 h after infusion. Plasma was separated and stored at −20°C until assay. Plasma concentrations of caffeine were determined by both GC and HPLC methods. Caffeine metabolites were determined by HPLC. Caffeine volume of distribution, elimination half-life, and total clearance were determined by previously described methods [19].

## RESULTS

### *Evaluation of the method*

Both the HPLC method (Fig. 1) and the GC method (Fig. 2) yielded well resolved, distinct peaks. Replicate samples ($n=6$) of each standard measured by HPLC (Table I) yielded coefficients of variation in an acceptable range. Calibration curves were linear and passed through the origin for all four compounds (Fig. 3.). The lower limit of HPLC detection for caffeine was 50 ng/ml and for each of the three metabolites 25 ng/ml. Between-day coefficients of variation determined from the slopes of the standard curves were 4.9, 8.1, 6.0 and 6.1%, respectively, for caffeine, theobromine, paraxanthine and theophylline.

Replicate samples ($n=6$) for caffeine measured by GC yielded coefficients of variation that ranged from 1.7 to 7.4% (Table I). Calibration curves were linear and passed through the origin. The lower limit of detection of caffeine was 5 ng/ml. Between-day coefficient of variation for GC-measured caffeine determined from the slope of the standard curves was 5.6%.

A total of 210 plasma caffeine concentrations were determined by both GC and HPLC methods (Fig. 4). The correlation coefficient was 0.986. The regression

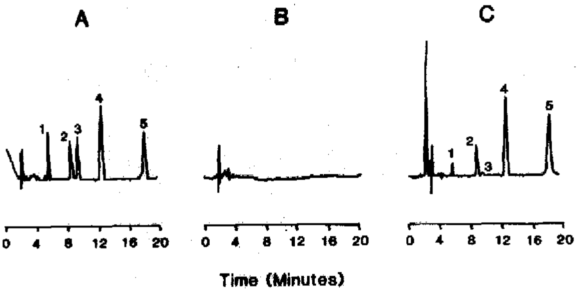


Fig. 1. Chromatographic tracings using the HPLC method. (A) Calibration standard; (B) subject blank; (C) sample from subject who ingested coffee on a daily basis. Peaks: 1 = theobromine; 2 = paraxanthine; 3 = theophylline; 4 = internal standard; 5 = caffeine.

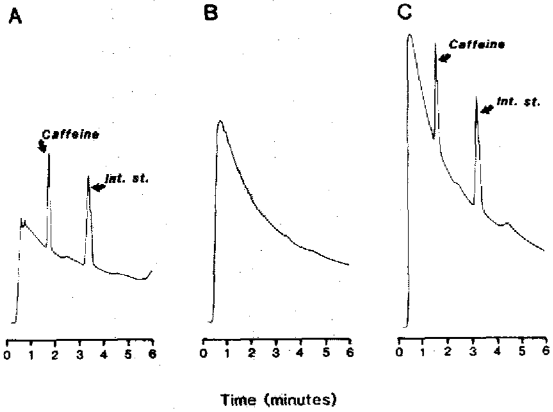


Fig. 2. Chromatographic tracings using the GC method. (A) Calibration standard; (B) subject blank; (C) sample from a subject following intravenous infusion of caffeine.

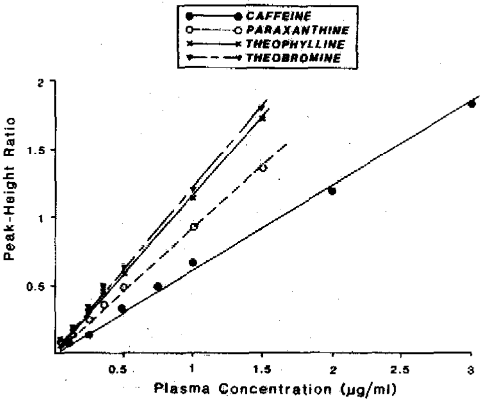


Fig. 3. Calibration curves from the HPLC method showing linear relation of plasma concentration of caffeine and its three metabolites to peak-height ratio of drug versus internal standard.

line slope was 1.02, and the intercept was 0.13. The coefficient of variation between methods was less than 15% for 75% of the samples. In 167 samples in which plasma caffeine concentration was 0.5 μg/ml or greater by both methods, the coefficient of variation between methods was less than 15% in 84.4% of the samples.

TABLE I

COEFFICIENTS OF VARIATION FOR IDENTICAL SAMPLES ANALYZED ON THE SAME DAY

At each concentration $n=6$. N.D. = non-detectable; — = not done.

| Plasma concentration (μg/ml) | Coefficient of variation (%) | | | | |
|---|---|---|---|---|---|
| | GC method | HPLC method | | | |
| | Caffeine | Caffeine | Paraxanthine | Theophylline | Theobromine |
| 0.005 | 6.5 | — | — | — | — |
| 0.010 | 4.5 | — | N.D. | N.D. | N.D. |
| 0.025 | 5.9 | N.D. | 9.3 | 8.7 | 4.9 |
| 0.05 | 3.1 | 8.1 | 7.9 | 5.8 | 9.6 |
| 0.1 | 5.0 | 2.9 | — | — | — |
| 0.125 | — | — | 3.0 | 5.0 | 9.0 |
| 0.25 | 1.9 | 2.1 | 4.0 | 3.0 | 5.0 |
| 0.5 | 3.0 | 5.0 | 2.0 | 5.0 | 5.5 |
| 0.75 | — | 2.5 | — | — | — |
| 1.0 | 1.7 | 1.0 | 2.0 | 2.9 | 6.0 |
| 2.5 | 7.4 | 2.5 | 1.3 | 1.0 | 3.0 |
| 5.0 | 4.0 | 1.9 | 7.0 | 7.0 | 4.5 |
| 10.0 | 2.9 | 6.3 | — | — | — |

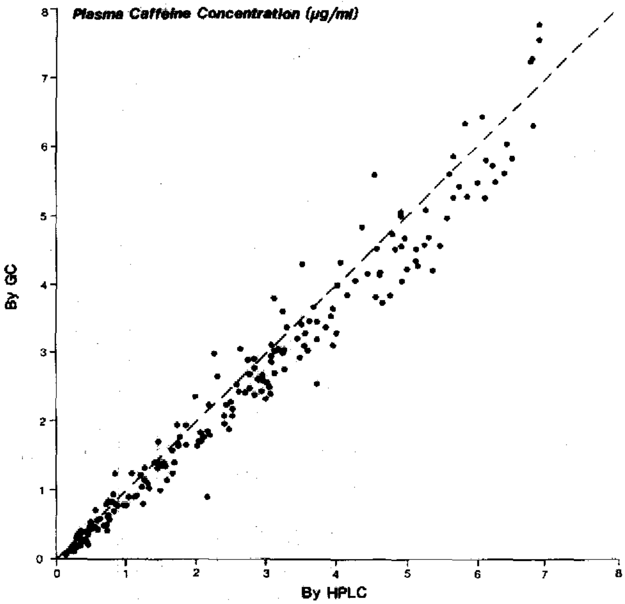
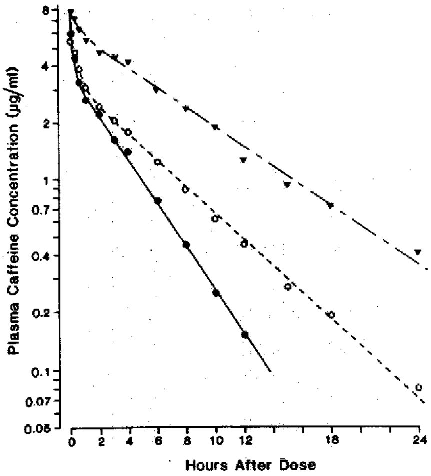


Fig. 4. Comparison of HPLC and GC determination of plasma caffeine concentrations in a series of samples. Dashed line is the line of identity ($y=x$). See text for method comparison analysis.



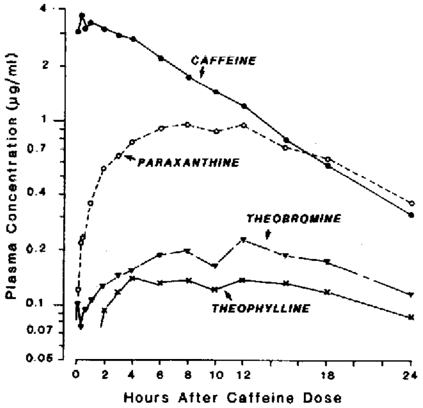


**Fig. 5. Plasma caffeine concentrations determined by the GC method in three representative subjects following intravenous infusion of caffeine.**

**Fig. 6. Plasma concentrations of caffeine and its three metabolites determined by the HPLC method in a representative subject following intravenous infusion of caffeine.**

The presence of sodium benzoate, acetaminophen, salicylic acid, ibuprofen and phenacetin did not interfere with either assay.

*Kinetic studies*

Fig. 5 shows the plasma concentration of caffeine measured by the GC method in representative subjects. Table II shows individual and mean kinetic variables for intravenous caffeine based on the GC method. Fig. 6 shows plasma concen-

TABLE II

RESULTS OF CAFFEINE PHARMACOKINETIC STUDY

| Subject No. | Weight (kg) | Volume of distribution (l/kg) | Elimination half-life (h) | Clearance (ml/min/kg) |
|---|---|---|---|---|
| 1 | 66.4 | 0.38 | 7.0 | 0.63 |
| 2 | 59.1 | 0.52 | 3.0 | 2.02 |
| 3 | 70.5 | 0.64 | 4.1 | 1.80 |
| 4 | 90.9 | 0.58 | 6.7 | 0.98 |
| 5 | 63.6 | 0.55 | 4.9 | 1.30 |
| 6 | 81.8 | 0.44 | 2.7 | 1.88 |
| 7 | 64.6 | 0.48 | 2.4 | 2.36 |
| 8 | 84.1 | 0.93 | 2.9 | 3.73 |
| 9 | 75.0 | 0.53 | 4.2 | 1.48 |
| 10 | 63.2 | 0.38 | 4.5 | 0.97 |
| 11 | 61.4 | 0.37 | 5.6 | 0.76 |
| Mean ± S.E. | 70.9 ± 3.2 | 0.53 ± 0.5 | 4.4 ± 0.5 | 1.63 ± 0.27 |

tration of caffeine and all three metabolites in a representative subject using the HPLC method.

## DISCUSSION

Quantitation of caffeine by HPLC is essential when caffeine metabolites must also be measured. We have described a GC method for measuring caffeine alone which is sensitive, specific and free of interference from other commonly encountered drugs. For the measurement of caffeine alone, the GC method is more sensitive than the HPLC method and requires significantly shorter preparation and run time. This GC method may be the method of choice for the screening of patients where caffeine concentrations alone are of interest or in single-dose pharmacokinetic studies of caffeine.

## ACKNOWLEDGEMENTS

We are grateful for the assistance of Nina Engelhardt and the staff of the Clinical Study Unit, New England Medical Center Hospital (supported by United States Public Health Service Grant RR-00054). This study was supported in part by Grants MH-34223 and AG-00106 from the United States Department of Health and Human Services. Dr. Kennedy was supported by the Alberta Heritage Medical Research Foundation, Alberta, Canada.

## REFERENCES

1 R. Hartley, J.R. Cookman and I.J. Smith, J. Chromatogr., 306 (1984) 191.
2 J. Blanchard, J.D. Mohammadi and K.A. Conrad, Clin.Chem., 26 (1980) 1351.
3 N.R. Scott, J. Chakraborty and V. Marks, J. Chromatogr., 375 (1986) 321.
4 B.R. Dorrbecker, S.H. Mercik and P.A. Kramer, J. Chromatogr., 336 (1984) 293.
5 R. Hartley, I.J. Smith and J.R. Cookman, J. Chromatogr., 342 (1985) 105.
6 F.L. Grab and J.A. Reinstein, J. Pharm. Sci., 57 (1968) 1703.
7 J.L. Cohen, C. Cheng, J.P. Henry and Y.-L. Chan, J. Pharm. Sci., 67 (1978) 1093.
8 H. Milon and J.A. Antonioli, J. Chromatogr., 162 (1979) 223.
9 I.D. Bradbrook, C.A. Jarnes, P.J. Morrison and H.J. Rogers, J. Chromatogr., 163 (1979) 118.
10 M. Bonati, D. Castelli, R. Latini and S. Garattini, J. Chromatogr., 164 (1979) 109.
11 E.E. Tyrala and W.E. Dodson, Arch. Dis. Child., 54 (1979) 787.
12 J.L. Brazier, D. Delaye, M. Desage and A. Bannier, J. Chromatogr., 224 (1981) 439.
13 F.T. Delbeke and M. Debackere, J. Chromatogr., 278 (1983) 418.
14 A. Locniskar and D.J. Greenblatt, J. Chromatogr., 374 (1986) 215.
15 R. Matlis and D.J. Greenblatt, J. Chromatogr., 310 (1984) 445.
16 M. Divoll, D.J. Greenblatt and R.M. Arendt, Int. J. Clin. Pharmacol., 22 (1984) 457.
17 D.J. Greenblatt, R. Matlis, D.R. Abernethy and H.R. Ochs, J. Chromatogr., 275 (1983) 450.
18 B. Ameer, D.J. Greenblatt, M. Divoll, D.R. Abernethy and L. Shargel, J. Chromatogr., 226 (1981) 224.
19 D.J. Greenblatt and J. Koch-Weser, N. Engl. J. Med., 293 (1975) 702, 964.