Journal of Chromatography, 568 (1991) 267–269
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5901

Short Communication

Elimination of caffeine interference in high-performance liquid chromatographic determination of cotinine in human plasma

S. PICHINI*, I. ALTIERI, R. PACIFICI, M. ROSA and P. ZUCCARO

Clinical Biochemistry Department, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome (Italy)

(First received January 16th, 1991; revised manuscript received March 13th, 1991)

ABSTRACT

A high-performance liquid chromatographic method with ultraviolet photometric detection for the determination of cotinine in human plasma was described. The use of a 30-cm reversed-phase column and of a mobile phase consisting of water–methanol–0.1 *M* sodium acetate–acetonitrile (72:21:5.6:1.4, v/v), pH 4.1, eliminated caffeine interference. A simplified solid-phase extraction procedure was also performed for plasma samples.

INTRODUCTION

Cotinine is one of the major metabolites of nicotine, and its determination in plasma or serum has been used to estimate daily nicotine intake from smoking and the exposure to tobacco smoke [1]. As cotinine has a long biological half-life, its plasma concentration is relatively stable throughout a day of smoking. Current assays for plasma cotinine are based on gas chromatography (GC) [2], gas chromatography–mass spectrometry (GC–MS) [3], high-performance liquid chromatography (HPLC) [4], and radioimmunoassay [5]. None of these reports mentioned the interference of caffeine, which is contained in some medications and drinks, and which can co-elute with cotinine.

A reversed-phase HPLC technique for urinary cotinine has been reported by Lequang Thuan *et al.* [6], requiring a lengthy liquid–liquid extraction. The resolution of peaks was not very high, so that cotinine and caffeine were not conveniently separated. Furthermore, this method could not assay plasma samples. We have optimized this method by using a 30-cm reversed-phase column and a new mobile phase to elute the analytes, thus eliminating caffeine interference. A simplified solid-phase extraction procedure was also performed for plasma samples, with ephedrine as an internal standard.

0378-4347/91/$03.50 © 1991 Elsevier Science Publishers B.V.

EXPERIMENTAL

Chemicals

Cotinine, caffeine and ephedrine were purchased from Sigma (St. Louis, MO, USA). Extrelut-1 extraction columns were obtained from Merck (Darmstadt, Germany). All solvents were analytical grade.

High-performance liquid chromatography

Chromatography was performed on a system equipped with a Merck-Hitachi L 6200 intelligent pump, a Merck-Hitachi L 4200 UV–VIS detector, a Merck-Hitachi D 2000 Chromato-Integrator, and a reversed-phase μBondapak C_{18} steel column (10 μm particle size, 30 cm × 4.6 mm I.D.; Waters, Milford, MA, USA).

The column was equilibrated with the mobile phase for at least 30 min prior to analysis of samples. The mobile phase was water–methanol–0.1 *M* sodium acetate–acetonitrile (72:21:5.6:1.4, v/v), was adjusted to pH 4.1 with acetic acid and used at a flow-rate of 1.4 ml/min. Ephedrine, cotinine and caffeine were detected at 254 nm.

Extraction procedure

A 0.5-ml aliquot of plasma, with 100 μl of ephedrine (25 μg/ml) and 0.5 ml of 5 *M* sodium hydroxide added, was introduced into a glass tube, shaken on a vortex mixer, and transferred to a pre-packed Extrelut-1 glass column, which was pre-conditioned with 8 ml of dichloromethane the day before. After 5 min, the analytes were eluted with 5 ml of dichloromethane–pentane (1:2, v/v). The organic phase, collected into a 5-ml glass tube, was evaporated to dryness under nitrogen and redissolved in 100 μl of water. A 20-μl volume was injected into the HPLC column.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram from a smoker's plasma containing 25 μg/ml ephedrine, 500 ng/ml cotinine and 500 ng/ml caffeine. The retention times were 4.49 min for ephedrine, 5.57 min for cotinine and 6.86 min for caffeine. The calibration curve for cotinine was linear over the range 10–500 ng/ml. Linear regression analysis gave a correlation coefficient of 0.98. The absolute recovery of the extraction ranged between 93 and 98%. The within-day and the between-day coefficients of variation (C.V.) were 3.8 and 7.2%, respectively, at a concentration of 200 ng/ml cotinine ($n = 12$). The limit of quantitation was 5 ng/ml (C.V. = 12%).

The separation of cotinine from caffeine was an essential result to obtain accurate quantification of plasma cotinine, because 80% of our samples contained high levels of caffeine. Furthermore, cotinine plasma levels, rather than urinary levels, may correlate with nicotine daily intake.

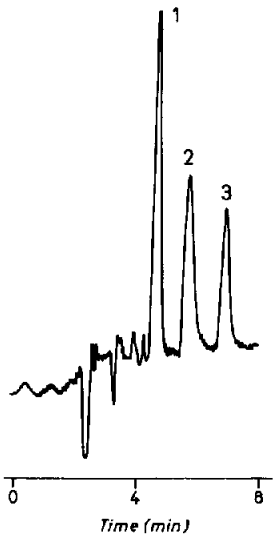

Fig. 1. Chromatogram from a smoker's plasma. Peaks: 1 = ephedrine (25 μg/ml); 2 = cotinine (500 ng/ml); 3 = caffeine (500 ng/ml).

ACKNOWLEDGEMENT

This study was supported in part by Grant No. 361 from Ministero delle Finanze, Monopoli di Stato.

REFERENCES

1 N. J. Holey, C. M. Axelrad and K. A. Tilton, *Am. J. Public Health*, 73 (1983) 1204.
2 P. Jacob, III, M. Wilson and N. L. Benowitz, *J. Chromatogr.*, 222 (1981) 61.
3 M. T. Parviainen, E. V. J. Puhakainen, R. Laatikainen, K. Savolainen, J. Herranen and R. D. Barlow, *J. Chromatogr.*, 525 (1990) 193.
4 A. Sioufi, C. Parisot, N. Sandrenan and J. P. Dubois, *Methods Find. Exp. Clin. Pharmacol.*, 11 (1989) 179.
5 J. J. Langone and H. Van Vunakis, *Methods Enzymol.*, 84 (1982) 628.
6 N. T. Lequang Thuan, M. L. Migueres, D. Roche, G. Roussel, G. Mahuzier, J. Chretien and O. G. Ekindjian, *Clin. Chem.* 35 (1989) 1456.