*Journal of Chromatography*, 622 (1993) 274–277
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 7143

# Short Communication

# Rapid determination of sparteine and its metabolites in urine

M. Veningerová*, V. Prachar and M. Lukácsová

*Institute of Preventive and Clinical Medicine, Limbová 14, 833 01 Bratislava (Slovak Republic)*

J. Glasa and J. Holomáň

*Department of Clinical Pharmacology, Postgraduate Medical Institute, Limbová 12, 833 02 Bratislava (Slovak Republic)*

(First received July 26th, 1993; revised manuscript received October 11th, 1993)

ABSTRACT

A method is presented for the isolation, separation and determination of sparteine and its metabolites in urine. The isolation is based on rapid extraction with dichloromethane and pentane in a glass separator. For the separation and determination, capillary gas chromatography with nitrogen–phosphorus detection was used. The recovery of the method ranged from 81.6% to 94.8%, and the limit of determination varied between 0.2 and 0.5 $\mu g\ ml^{-1}$. For quantification, 17-ethylsparteine was used as the internal standard.

## INTRODUCTION

Pharmacogenetics has revealed several types of metabolic polymorphism of xenobiotics. The oxidative polymorphism of the debrisoquine/sparteine type is one of the most frequently investigated [1–4].

Sparteine, which is primarily used as an antiarrhythmic and uterotonic acting drug, is metabolized via N-oxidation by cytochrome P450 in microsomal enzymatic liver fractions. It yields two main metabolites, 2-dehydrosparteine and 5-dehydrosparteine (DHS), which, together with the parent compound, are excreted in the urine.

According to different studies [1,6–8], the frequency of the defective N-oxidation of sparteine in the white population (poor metabolizer, PM) varies from 4% to 9%. Criteria for classification of metabolizers on the basis of metabolic ratio (MR) were reported by Eichelbaum [7] and others [8]. Eichelbaum *et al.* [1] have also reported on the genetic predetermination of this type of polymorphism. This resulted in increased interest in the testing of metabolic phenotype by sparteine or other drugs, *e.g.* debrisoquine and dextromethorphan. They can serve as metabolic markers in drugs and other xenobiotics.

The published methods for determining sparteine and its metabolites in biological materials include gas chromatography [9–12], thin-layer chromatography [13], and high-performance liq-

* Corresponding author.

0378-4347/93/$06.00 

uid chromatography [14]. The isolation was based mainly on extraction with dichloromethane from an alkaline medium. Because of the low stability of the sparteine metabolites in strongly basic media, some authors [11,12] have described their reduction by sodium borohydride to the parent compound, and the determination of sparteine before and after reduction.

A rapid, efficient and reliable method for the isolation of sparteine and its metabolites was the aim of this study. Extraction with dichloromethane–pentane in a glass separator [5] and the final determination by capillary GC with nitrogen–phosphorus detection was found to be the most suitable method. The determination of the oxidation phenotype in a group of 252 volunteers from the Slovak population shows the practical application of this method.

## EXPERIMENTAL

### *Chemicals*

Sparteine (7,14-methano-2*H*-dipyrido[1,2-a:1′,2′-e][1,5]diazocine), available from Sigma (Prague, Czech Republic), 5-dehydrosparteine (5-DHS), 2-dehydrosparteine (2-DHS), and 17-ethylsparteine (17-ES) were a generous gift from Dr. M. Eichelbaum (Dr. Margarete Fischer-Bosch Institute, Stuttgart, Germany). Dichloromethane, pentane and sodium hydroxide were purchased from E. Merck (Darmstadt, Germany).

### *Apparatus*

The gas chromatographic system consisted of a Hewlett-Packard Model 5890 A chromatograph equipped with a nitrogen–phosphorus detector, an HP Model 3393 integrator and an HP Model 7673 autosampler.

### *Separation conditions*

A fused-silica capillary column (25 m × 0.33 mm I.D., 0.20 $\mu$m film thickness) containing HP-1 (methyl silicone gum) was used. The column temperature was initially 70°C (for 0.5 min), then increased at 40°C $min^{-1}$ to 190°C; it was held here for 1 min, then increased again at 2°C $min^{-1}$ to 200°C; this final temperature was held for 5 min. Other conditions: carrier gas, nitrogen; column head-pressure, 60 kPa; detector temperature, 250°C; hydrogen flow-rate, 3 ml $min^{-1}$; air flow-rate, 54 ml $min^{-1}$; make-up gas, nitrogen at a flow-rate of 30 ml $min^{-1}$; injector temperature, 230°C. All injections were made in the splitless mode.

### *Standard stock solutions*

Solutions of sparteine sulphate monoperchlorate (100 $\mu$g $ml^{-1}$), 5-dehydrosparteine monoperchlorate (131 $\mu$g $ml^{-1}$), and 2-dehydrosparteine monoperchlorate (146 $\mu$g $ml^{-1}$) were prepared separately in 0.1 *M* HCl.

### *Extraction of sparteine and its metabolites*

Sparteine and its metabolites were analysed by a modified method according to Eichelbaum *et al.* [1] using the extraction of sparteine and its metabolites in a solvent glass separator. A 5-ml volume of a cooled urine sample (5°C) was placed in a 14-cm length extraction tube with a reversed conical joint (Fig. 1). Then 100 $\mu$l of 10 *M* NaOH and 50 $\mu$l of the stock solution of

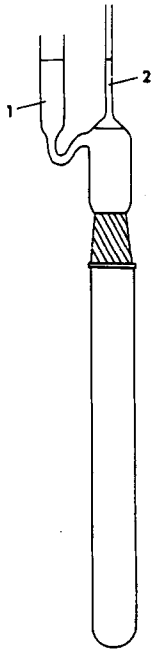


Fig. 1. Separator for the extraction of sparteine and dehydrosparteines from urine [5]: 1 = side-arm for water; 2 = capillary for extract.

17-ethylsparteine (10 $\mu$g ml$^{-1}$) were added, followed by 1 ml of pentane–dichlormethane (1:1, v/v). The sample was then mixed on a vortex-mixer. After separation of the layers, the separator was attached to the extraction tube and distilled water was added through the side-arm, until the organic phase was transferred into the capillary for the extract. Immediately, 1 $\mu$l of the extract was taken into a glass microvial and injected into the GC injector (Fig. 2).

*Calibration*

Calibration curves for sparteine, 5-dehydrosparteine, and 2-dehydrosparteine were established in the range from 0.5 to 10 $\mu$g ml$^{-1}$, using working standards prepared in 0.1 *M* HCl. These standards were subjected to the same treatment as the real urine samples. Blanks containing the internal standard (17-ethylsparteine) were analysed in the same way.

All calculations were based on the ratio of the peak areas of sparteine and the two dehydrosparteines to the peak area of the internal standard.

*Sample collection*

The urine samples were collected in glass vials 12 h after an oral administration of 100 mg of sparteine (sparteine sulphate pentahydrate), and immediately deep frozen. The volunteers were chosen from the normal population, in the age range 19–60.

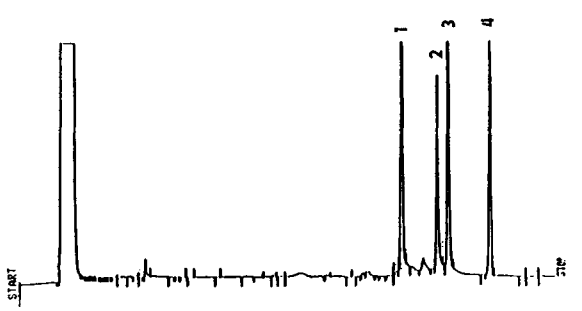


Fig. 2. Chromatogram of urine spiked with standard mixture sparteine (1), 5-dehydrosparteine (2), 2-dehydrosparteine (3), and 17-ethylsparteine (4), obtained with an HP-1 fused-silica capillary column.

## RESULTS AND DISCUSSION

Because of the specific biotransformation pathway properties of sparteine and its metabolites [1,11,12], an efficient method for their isolation and identification from urine is required. A rapid method for the isolation using extraction in a modified separator [5] was developed. The advantage of this procedure is a considerably shorter isolation time than those of published methods. No emulsion is formed, and the extract can be directly analysed by capillary GC.

The evaluation of the concentrations of 2- and 5-DHS showed that the content of 2-DHS in urine samples was four to five times higher than that of 5-DHS, which is in good correlation with the data published by Eichelbaum [1].

For the quantification of sparteine metabolites, especially in poor metabolizers, who excrete less than 2% of the sparteine dose in the form of 2- and 5-dehydrosparteine within 12 h, the given limit of determination of the method can be a source of difficulties. The described separator solves this problem to some extent, because it makes it possible to increase the volume of the urine sample to 15 ml.

Table I presents the recoveries of sparteine and its metabolites from spiked urine in the concentration range 0.5–10 $\mu$g ml$^{-1}$. The limits of determination were: sparteine and 17-ethylsparteine, 0.2 $\mu$g ml$^{-1}$; 5-DHS, 0.5 $\mu$g ml$^{-1}$; 2-DHS, 0.4 $\mu$g ml$^{-1}$. The recovery of 17-ethylsparteine was 91.8% (S.D. ± 8.5%).

For determining the within-series precision, six parallel determinations of two urine samples, containing different concentrations of sparteine and dehydrosparteines, were carried out. The standard deviations are listed in Table I.

The metabolic ratio (MR) was calculated from the results of 252 investigations. In this pool, the frequency distribution (Fig. 3) shows that most log MR values are in the range 0.4–0.01. This is in good correlation with the results of other authors [6,10].

Apart from the necessity to determine the genetic polymorphism of the metabolism of xenobiotics with respect to the investigation of the effectiveness and/or safety or drugs, an

TABLE I

RECOVERIES OF SPARTEINE AND ITS METABOLITES FROM URINE

| Compound | Concentration (mean ± S.D., $n = 6$) ($\mu$g ml$^{-1}$) | | Recovery (%) |
|---|---|---|---|
| | Added | Found | |
| Sparteine | 10.0 | 9.29 ± 0.82 | 92.90 ± 8.26 |
| | 1.0 | 1.02 ± 0.24 | 102.00 ± 2.42 |
| | 0.5 | 0.46 ± 0.01 | 92.00 ± 0.82 |
| 5-DHS | 1.31 | 1.06 ± 0.18 | 80.91 ± 14.10 |
| | 0.66 | 0.67 ± 0.00 | 101.83 ± 7.20 |
| 2-DHS | 1.46 | 1.27 ± 0.20 | 86.98 ± 13.57 |
| | 0.73 | 0.67 ± 0.00 | 91.78 ± 8.50 |

important aspect is the development of ecogenetics. This deals with the relationship of environmental contaminants to genetic polymorphism. In this connection, it is necessary to study the function of the liver directly in the human organism in relation to the contaminants investigated (pesticides, industrial chemicals of PCB-type, polychlorinated benzenes, phenols, dioxines, furanes, etc.) and their potentially adverse effects. Sparteine seems to be suitable for this purpose as one of the markers of the functional diagnostics of liver.

ACKNOWLEDGEMENT

The authors thank Prof. Dr. M. Eichelbaum for valuable help in introducing the analytical method, as well as for the standards.

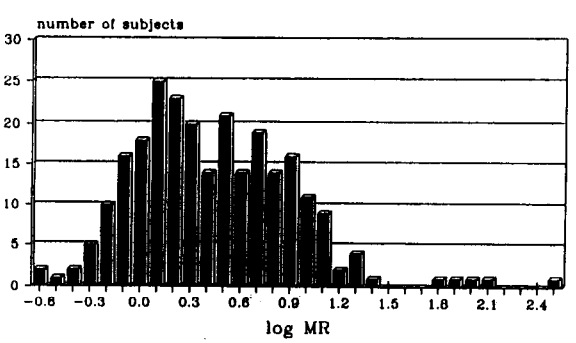


Fig. 3. Frequency distribution of the oxidation phenotype in the Slovak population.

REFERENCES

1 M. Eichelbaum, K.P. Reetz, E.K. Schmidt and C. Zekorn, *Xenobiotica*, 16 (1986) 465.
2 B.A. Osikowska-Evers and M. Eichelbaum, *Life Sci.*, 38 (1986) 1775.
3 D.A.P. Evans, D. Harmer, D.Y. Downham, E.J. Whibley, J.R. Idle, J. Ritchie and R.L. Smith, *J. Med. Genetics*, 20 (1983) 321.
4 M. Van Der Graaff, N.P.E. Vermeulen, M.C. Koperdraat and D.D. Breimer, *Arch. Int. Pharmacodyn.*, 282 (1986) 181.
5 J. Hrivnák, *Anal. Chem.*, 57 (1985) 2159.
6 T. Ishizaki, M. Eichelbaum, Y. Horai, K. Hashimoto, K. Chiba and H.J. Dengler, *Br. J. Clin. Pharmacol.*, 23 (1987) 482.
7 M. Eichelbaum, *ISI Atlas of Science: Pharmacology*, (1988) 243.
8 W.D. Paar, H. Schuhler, R. Fimmers and H.J. Dengler, *Eur. J. Clin. Pharmacol.*, 36 (1989) 555.
9 M. Eichelbaum, personal communication.
10 J. Pospíšil, F. Perlík, V. Patzelová and V. Kordač, *Biochem. Clin. Bohemoslov.*, 19 (1990) 343.
11 J.H.M. Schellens, H.H.F. Van Der Wart, J.W. Hoevers and D.D. Breimer, *J. Chromatogr.*, 431 (1988) 203.
12 T. Inaba, A. Vinks and S.V. Otton, *J. Clin. Pharmacol.*, 21 (1986) 473.
13 T. Ebner, C.O. Meese and M. Eichelbaum, *Ther. Drug Monit.*, 11 (1989) 214.
14 J. Moncrieff, *J. Chromatogr.*, 529 (1990) 194.