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Note

Determination of the two major metabolites of ebselen in human plasma by high-performance liquid chromatography

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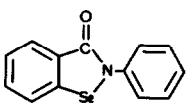
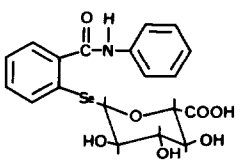
Ebselen (Fig. 1), a highly tolerable organoselenium compound, shows an anti-inflammatory profile in various experimental models which is different to that of other non-steroidal anti-inflammatory agents. Owing to its glutathione peroxi-

Ebselen



I



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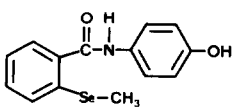
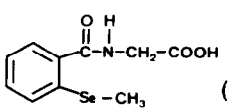


III



(I.S.)

Fig. 1. Structures of ebselen, its main metabolites I and II and the internal standard III.

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dase-like activity, ebselen appears to be capable of degrading reactive oxygen species which are considered to be potent inflammatory mediators.

Following oral administration to man and animals, ebselen is readily absorbed and hepatic transformation into phase I and II metabolites rapidly occurs [1,2]. Unchanged ebselen has never been found in human plasma. The major metabolites are a novel selenoglucuronide I and a selenomethyl compound II (Fig. 1). Especially the pharmacokinetic behaviour of the selenoglucuronide, the first ever described, is of great importance for the further development of substances like ebselen.

In this paper, an efficient high-performance liquid chromatographic (HPLC) assay for the determination of the two metabolites using solid-phase extraction is described.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent or higher grade and aqueous solutions were prepared in doubly distilled water. They included methanol and ethanol (Riedel-de Haën, Hannover, F.R.G.), acetonitrile (HPLC grade S; Rathburn Chemicals, Walkerburn, U.K.), orthophosphoric acid (Fluka, Buchs, Switzerland) and citrate–hydrochloric acid buffer (pH 2.0) (Puffer-Titrisol, Art. No. 9882; Merck, Darmstadt, F.R.G.). The glucuronide I (purity 97%), the selenomethyl compound II (purity 97%) and the internal standard III (I.S.) (purity > 97%) (Fig. 1) were synthesized in our laboratories.

Sample preparation

All plasma samples were centrifuged before use.

In a glass tube, 1.5 ml of plasma were mixed with the internal standard (20 μl of ethanol containing 5 μg of III), acidified with hydrochloric acid (1 mol/l; 200 μl) and citrate–hydrochloric acid buffer (500 μl; pH 2.0) and briefly vortexed. The mixture was loaded onto an octadecylsilane bonded silica column (Bond-Elut C_{18}, 3-ml capacity; Analytichem International, Harbor City, CA, U.S.A.). The column was conditioned immediately prior to use by washing with 2 ml of water, 2 ml of acetonitrile and 2 ml of hydrochloric acid (0.01 mol/l).

The sample was passed through the column and then washed with 2 ml of hydrochloric acid (0.01 mol/l). Residual water was removed from the column as completely as possible by applying pressure before eluting with 1.5 ml of acetonitrile. The eluent was evaporated to dryness in a vacuum centrifuge at 40°C (Speed Vac concentrator, Savant Instruments, Hicksville, NY, U.S.A., distributed by UniEquip, Martinsried, F.R.G).

The residue was dissolved in 80% methanol (200 μl) and an aliquot (10 μl) was injected into the HPLC system and analysed.

Chromatography

The HPLC separations were carried out using a Hewlett-Packard high-performance liquid chromatograph with a UV filter detector (Model 1090) equipped

with a Waters 820 chromatography data station for data evaluation. The analytical column was a Nucleosil C_{18} (Macherey & Nagel, Düren, F.R.G.), particle size 7 μm (250 mm × 4 mm I.D.). The mobile phase included (A) acetonitrile and (B) 0.1% phosphoric acid in water (pH 2.5). Chromatography was performed at 40°C with a flow-rate of 1.5 ml/min. The eluent was monitored at 254 nm. A linear gradient from 10 to 30% B in 14 min was used to elute the internal standard III and the glucuronide I. Subsequently the proportion of B was increased to 50% in 10 min to elute II. To clean the column the proportion of B was increased to 95% in 2 min and held there for 3 min. The column was equilibrated for 3 min after each run.

Preparation of calibration graph

The stock solution for I and II was prepared in ethanol at a concentration of 1 g/l and stored at 4°C.

Calibration graphs were prepared by adding I and II to drug-free samples of fresh human plasma to give a concentration range from 0.3 to 13.3 mg/l. These standards were processed according to the assay procedure. The ratios of the peak areas of both I and II to that of III (I.S.) were used to construct the calibration graphs.

RESULTS AND DISCUSSION

The chromatograms of blank plasma and a plasma sample spiked with 3.3 mg/l each of I, II and III as internal standard are shown in Fig. 2A and B. No significant interference was observed at the retention times of the three compounds.

III proved to be very suitable as an internal standard, having similar extraction properties and chromatographic characteristics to I and II.

The calibration graphs of peak-area ratio versus concentration were linear over the concentration range tested. The best-fit curve was obtained using linear regression analysis. The result for I was $y = 0.253\ (\pm 0.014) + 1.886(\pm 0.017)x$ with a correlation coefficient of $r = 0.999$ ($n = 4$). The equation for II was $y = 0.095\ (\pm 0.018) + 1.160(\pm 0.012)x$ with a correlation coefficient of $r = 0.999$ ($n = 4$). The values in parentheses represent the standard deviation for the slope and the y-intercept. The within-day precision for each compound was determined by analysis of four or five identically spiked plasma samples at seven concentrations (Table I). The detection limit was 0.05 mg/l in plasma (signal-to-noise ratio $\geqslant 3$).

The recoveries for I and II (and also III) from plasma were in the range 87–100% (Table II).

The application of the method was demonstrated by measuring the plasma levels of I in human volunteers after single oral doses of ebselen. Fig. 2C shows a chromatogram of plasma obtained from a volunteer 2 h after administration of 1000 mg of ebselen. The peak with an elution time 12.55 min (I) corresponds to 4.1 mg/l and that at 18.14 min (II) corresponds to 0.7 mg/l. The results of this study with human volunteers will be published elsewhere.

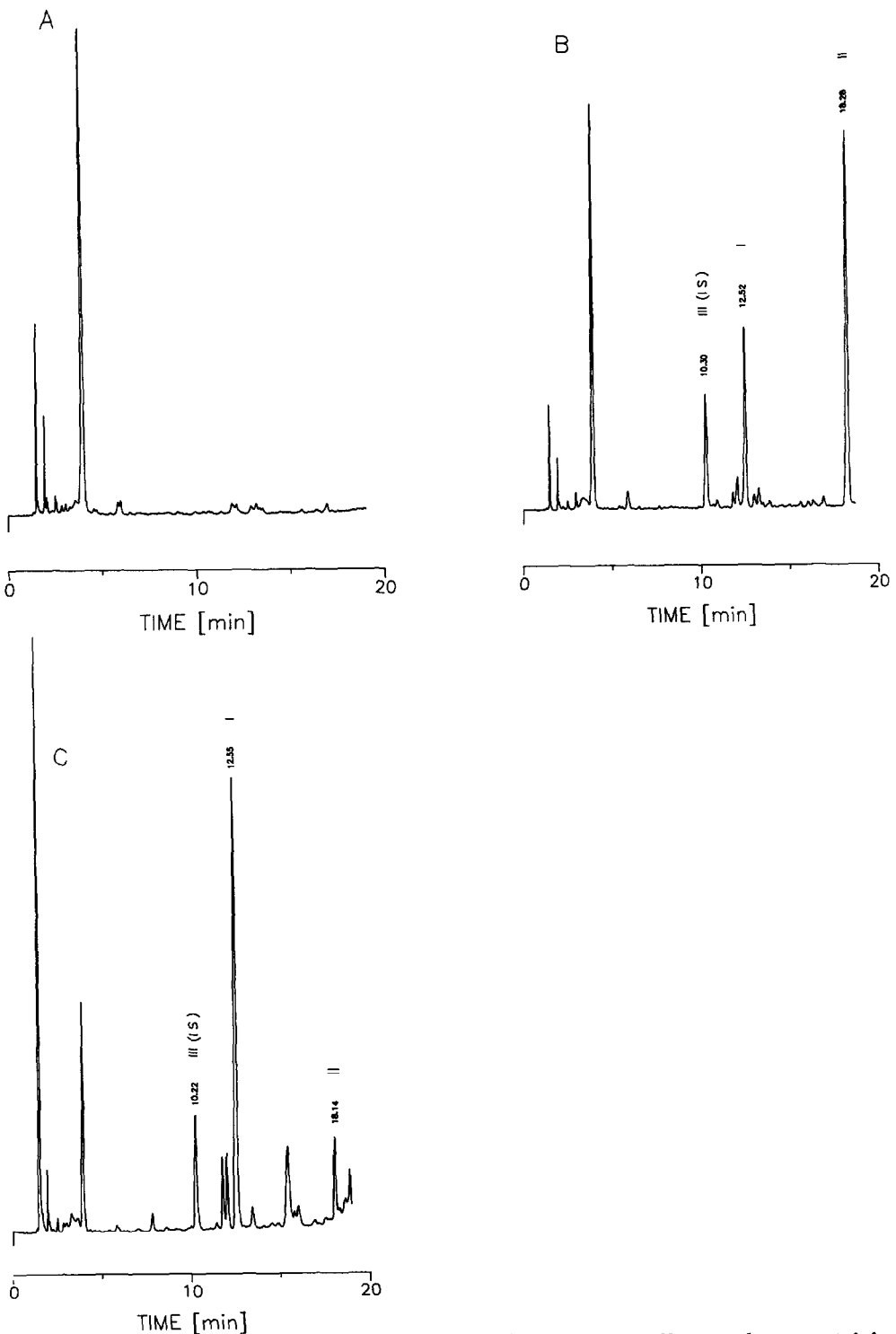

Fig. 2. (A) Chromatogram of blank human plasma. (B) Chromatogram of human plasma containing 3.3 mg/l each of I, II and III as internal standard. (C) Chromatogram of plasma obtained from a volunteer 2 h after oral administration of 1000 mg of ebselen.

TABLE I

WITHIN-DAY PRECISION OF THE METHOD

Coefficients of variation (C.V.) for four or five samples were calculated from the standard deviation of the arithmetic mean of the peak-area ratio at each concentration.

Concentration (mg/l)	n	C.V. (%)	
		I	II
0.33	5	1.85	1.21
0.67	5	1.98	1.79
2.00	5	3.11	1.22
3.33	5	1.33	1.22
5.33	4	1.94	0.89
10.00	4	2.44	0.70
13.33	4	1.83	1.23

TABLE II

RECOVERIES OF I, II AND III FROM HUMAN PLASMA

Concentration (mg/l)	Recovery (%)		
	I	II	III
0.33	95 (n=6)	96 (n=4)	
2.00	87 (n=8)	92 (n=4)	
3.33			95 (n=11)
5.33	92 (n=7)	97 (n=4)	
13.33	97 (n=7)	99 (n=4)	

CONCLUSIONS

The assay described proved to be suitable for the determination of the two major metabolites of ebselen in human plasma. The sensitivity is sufficient for pharmacokinetic studies in man.

REFERENCES

1 R. Terlinden, H. Fischer and A. Römer, poster presented at the 3rd European Congress of Biopharmaceutics and Pharmacokinetics, Freiburg, 1987 (Congress Abstract No. 216).
2 A. Müller, H. Gabriel, H. Sies, R. Terlinden, H. Fischer and A. Römer, Biochem. Pharmacol., 37 (1988) 1103.