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Note

Gas chromatographic determination of two fluorinated benzodiazepines in rats and mice

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7-Chloro-1,3-dihydro-1-hydroxyethyl-3-hydroxy-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one (SAS 643) and 7-chloro-1,3-dihydro-1-methyl-3-hydroxy-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one (SAS 646) differ in the N_1 substitution (Fig. 1). SAS 643 has sedative and sleep-inducing effects in rats, while SAS 646 possesses prevalently an anxiolytic activity[1–3].

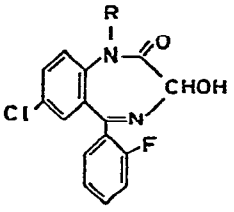

Fig. 1. Structural formulae of SAS 643 (R = CH_2–CH_2–OH) and SAS 646 (R = CH_3).

This paper describes a very sensitive gas chromatographic (GC) method to measure the levels of SAS 643 and SAS 646 in biological samples from rats and mice. The two compounds can be detected without any derivatization, but with poor sensitivity because of their polar groups. We have therefore derivatized the molecules in order to obtain better sensitivity, in the nanogram range.

EXPERIMENTAL

Gas chromatographic conditions

The gas chromatograph was a Fractovap Model 215 (Carlo Erba, Milan, Italy) equipped with a ^{63}Ni electron capture detector. The stationary phase was 3% OV-17 on Gas-Chrom Q (100–120 mesh) packed into a silylated glass column (1 m × 4 mm I.D., 6 mm O.D.). The most favourable analytical conditions for SAS 643 and SAS 646 were: column temperature, 285°C; detector temperature, 305°C; injection port temperature, 310°C; gas (nitrogen) flow-rate; 30 ml/min; purge gas (nitrogen) flow-rate, 35 ml/min.

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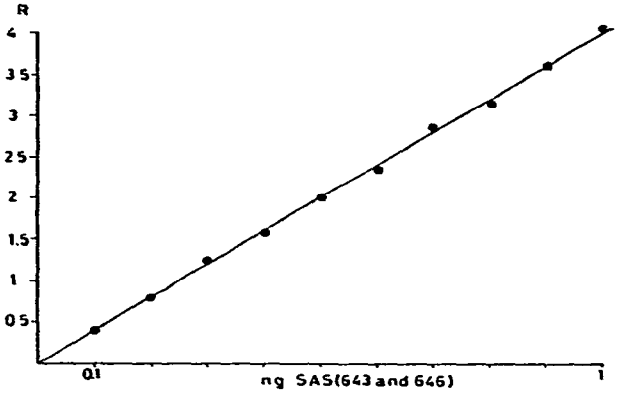
Quantitative analysis

Serial amounts of the silyl derivatives of SAS 643 and SAS 646 dissolved in an acetonitrile solution of the internal standard [2,4-dinitrophenylhydrazone (DNPH) of camphor, 2 ng/μl in acetone] were injected into the GC column. A linear relationship was found between the relative peak areas and concentrations of both drugs in the 0.1–1 ng/μl range (Fig. 2). Fig. 3 shows a typical gas chromatogram obtained by injecting SAS 643 and the internal standard. SAS 646 injection gives an identical picture.



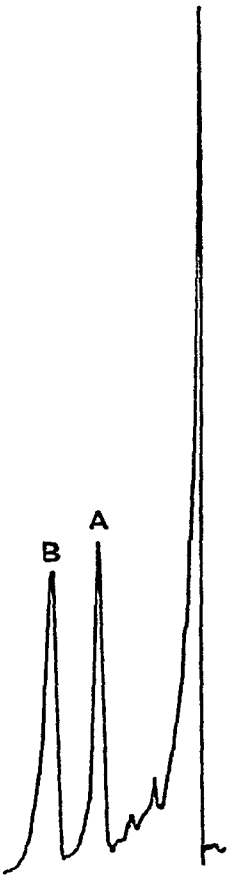


Fig. 2. Calibration curve of SAS 643 and SAS 646. R = area SAS (643 and 646) / area internal standard.

Fig. 3. Gas chromatogram of SAS (2 ng/μl) (A) and its internal standard (20 ng/μl DNPH) (B).

The identity of the GC peaks of the silyl derivatives of SAS 643 and SAS 646 was checked by mass spectrometry (MS) coupled with GC. Analysis of the spectrum shows that the silylated compounds correspond to known structures and are not modified during gas chromatography.

Animals and drug administration

Male CD COBS rats, body weight 200 g, and male CD_1 mice, body weight 22 ± 3 g, were purchased from Charles River Italy (Calco, Italy). For intravenous injection the drugs were dissolved in a mixture of diethylacetamide, Tween 80 and water (0.1:1:3.9). The dose was 5 mg/kg in 0.2 ml. Controls were given the same volume of the solvent.

For oral administration, drugs were homogenized in a glass-PTFE potter homogenizer in a solution of 5% carboxymethylcellulose in water. The dose was 5 mg/kg. At different times after drug administration the animals were killed and their blood and tissues were collected.

Extraction from blood

To 1 ml of heparinized blood were added 3 ml of benzene; the sample was shaken for 3 min and kept at room temperature for 5 min to ensure good separation of the two phases. A 2-ml volume of extract was dried under vacuum; to the residue, taken up with 75 μl of the acetonitrile solution of the internal standard (DNPH of camphor), were added 25 μl of N,O-bis(trimethylsilyl)acetamide (BSA) (BDH, Poole, Great Britain). The sample was stirred for 1 min, left for 5 min and then injected into the GC column.

For studies of recovery, 30 ng of the two compounds dissolved in 10 μl of acetone were added to 1 ml of blood from untreated rats or mice. Recovery in both cases was 98 ± 2%.

Extraction from tissues

Brain and epididymal adipose tissue were homogenized in an all-glass potter homogenizer with 0.1 *M* glycine buffer pH 10.2 (1:4, w/v). Under agitation, 1 ml of homogenate was withdrawn and mixed with 3 ml of benzene; after stirring, the samples were spun at 3020 *g* for 5 min. The procedure from this point was as for plasma.

For studies of the recovery, 60 ng of the two drugs dissolved in 10 μl of acetone were added to homogenized tissues: brain recovery of 97% and an epididymal tissue recovery of 70% was obtained for both drugs. Pharmacokinetic parameters were calculated using the NONLIN program[4] on a Univac 1103 digital computer.

RESULTS AND DISCUSSION

Intravenous administration to rats

Table I shows the distribution of SAS 643 and SAS 646 in rat blood, brain and adipose tissue at intervals after intravenous injection of 5 mg/kg. SAS 643 blood and brain levels were initially comparable, after which the drug disappeared more rapidly from brain, where it had become undetectable after 2 h. Levels in adipose tissue were already higher at 15 min, which means distribution takes place in less than 15 min. The drug, which is liposoluble, accumulated in adipose tissue and the concentrations were still very high even after 6 h.

Brain levels of SAS 646 (Table I) were always greater than those in blood and the area under the concentration *versus* time curve (AUC) for brain was about 5 times that for the mouse (see also Table IV). SAS 646 did not accumulate in adipose, the levels in this tissue being always much lower than in brain.

TABLE I

DISTRIBUTION OF SAS 643 AND SAS 646 IN RAT BLOOD, BRAIN AND ADIPOSE TISSUE AFTER I.V. INJECTION OF 5 mg/kg

Each value is the mean of four determinations.

Time after administration	*Blood*		*Brain*		*Adipose tissue*	
	μg/ml ± S.E.		*μg/g ± S.E.*		*μg/g ± S.E.*	
	SAS 643	*SAS 646*	*SAS 643*	*SAS 643*	*SAS 643*	*SAS 646*
15 min	1.57 ± 0.25	1.08 ± 0.06	1.44 ± 0.16	8.42 ± 0.05	13.20 ± 0.85	2.09 ± 0.01
30 min	1.26 ± 0.13	0.67 ± 0.05	0.93 ± 0.10	7.39 ± 0.10	6.20 ± 0.45	0.83 ± 0.08
1 h	0.35 ± 0.05	0.19 ± 0.03	0.31 ± 0.03	4.00 ± 0.03	3.40 ± 0.31	0.51 ± 0.05
2 h	0.02 ± 0.01	0.01 ± 0.001	0.03 ± 0.008	0.26 ± 0.001	1.79 ± 0.25	0.02 ± 0.02
6 h	0.005 ± 0.001	< 0.002	< 0.002	0.07 ± 0.01	0.56 ± 0.05	< 0.005

Intravenous administration to mice

Table II shows the levels of SAS 643 and SAS 646 in mouse blood and brain after i.v. injection of 5 mg/kg. The brain accumulated less SAS 643 and eliminated it more slowly.

There were some differences between rat and mouse in the distribution of the drug. Levels are always higher in mice, especially in blood, and the disappearance of SAS 643 is slower in mice: the half-life of the drug is 0.29 h in rat blood and 1.32 h in mouse blood. As previously described for rat, SAS 646 levels in brain were always greater than those in blood and the AUC brain/AUC blood ratio for the mouse was about 2.

TABLE II

LEVELS OF SAS 643 AND SAS 646 IN MOUSE BLOOD AND BRAIN AFTER INTRAVENOUS INJECTION OF 5 mg/kg

Each sample was the pooled specimens from four mice and each value is the mean of four samples.

Time after injection	*Blood*		*Brain*	
	μg/ml ± S.E.		*μg/g ± S.E.*	
	SAS 643	*SAS 646*	*SAS 643*	*SAS 646*
5 min	4.49 ± 0.40	—	2.34 ± 0.20	—
30 min	2.76 ± 0.20	0.76 ± 0.01	2.24 ± 0.20	1.61 ± 0.04
1 h	1.66 ± 0.20	0.27 ± 0.01	0.77 ± 0.05	0.42 ± 0.01
3 h	0.15 ± 0.03	0.03 ± 0.002	0.21 ± 0.002	0.03 ± 0.007
6 h	0.05 ± 0.007	0.01 ± 0.002	0.01 ± 0.001	0.02 ± 0.003
12 h	0.008 ± 0.002	—	< 0.005	—

Oral administration to rats

Table III shows the levels of SAS 643 and SAS 646 in rats after oral administration of 5 mg/g. The drug distribution was qualitatively the same as after i.v. treatment: SAS 643 was rapidly absorbed (reaching peak levels after 0.5 h) and disappeared rapidly from blood, accumulating in adipose tissue. Brain levels were always higher (nearly double) than blood concentrations.

TABLE III

DISTRIBUTION OF SAS 643 AND SAS 646 IN RAT BLOOD BRAIN AND ADIPOSE TISSUE AFTER ORAL ADMINISTRATION OF 5 mg/kg

Each value is the mean of four determinations, four rats being used for each determination.

Time after administration	*Blood*		*Brain*		*Adipose tissue*	
	μg/ml ± S.E.		*μg/g ± S.E.*		*μg/g ± S.E.*	
	SAS 643	*SAS 646*	*SAS 643*	*SAS 646*	*SAS 643*	*SAS 646*
15 min	0.05 ± 0.005	0.03 ± 0.004	0.09 ± 0.012	0.05 ± 0.012	0.34 ± 0.03	0.07 ± 0.008
30 min	0.06 ± 0.007	0.05 ± 0.013	0.08 ± 0.008	0.05 ± 0.017	0.45 ± 0.05	0.10 ± 0.014
1 h	0.06 ± 0.006	0.05 ± 0.114	0.11 ± 0.005	0.09 ± 0.020	0.29 ± 0.02	0.08 ± 0.005
2 h	0.02 ± 0.005	0.03 ± 0.002	0.05 ± 0.003	0.05 ± 0.016	0.59 ± 0.08	0.06 ± 0.006
3 h	0.02 ± 0.003	0.01 ± 0.001	0.02 ± 0.004	0.02 ± 0.003	0.11 ± 0.01	0.04 ± 0.006
4 h	0.02 ± 0.004	< 0.002	0.002 ± 0.001	< 0.002	0.08 ± 0.01	0.03 ± 0.001

SAS 646 brain levels after oral administration were higher than the blood levels, but always lower than those in adipose tissue. Oral administration gave considerably lower SAS 646 levels in all tissues than those after i.v. administration at the same dose.

The results in Table I and II were subjected to mathematical analysis and the resulting pharmacokinetic parameters are set out in Table IV.

TABLE IV

PHARMACOKINETIC PARAMETERS CALCULATED ON THE DATA IN TABLES I AND II

AUC = Area under the concentration *versus* time curve (μg·h/ml);
$T^1/_2$ = half-life of elimination phases (h); k_{el} = rate constant of elimination (h^{-1}).

	AUC		$T^1/_2$		k_{el}	
	SAS 643	*SAS 646*	*SAS 643*	*SAS 646*	*SAS 643*	*SAS 646*
Rat						
Blood	1.06	0.85	0.71	0.43	0.98	1.60
Brain	1.21	10.15	0.32	0.79	2.18	0.87
Adipose tissue	16.45	153.84	1.47	0.43	0.47	1.61
Mouse						
Blood	4.46	0.85	1.32	0.93	0.52	0.74
Brain	2.88	1.36	0.75	0.91	0.92	0.75

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