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Determination of Sultopride in Serum and Saliva by High-Performance Liquid Chromatography

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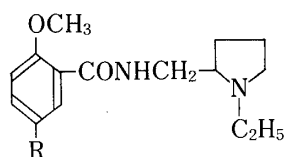
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A high-performance liquid chromatographic (HPLC) method for the determination of sultopride in serum and saliva was developed. The limit of detection of sultopride using 0.5 ml sample volume was $0.04 \mu\text{g/ml}$ in serum and saliva. The recoveries of sultopride added to serum and saliva ($2 \mu\text{g/ml}$) were $99.6 \pm 3\%$ and $99.8 \pm 3\%$ (mean \pm S.D., $n=5$), respectively. The coefficients of variation of within-run assays for 3 levels of sultopride in serum were less than 4%. There was good agreement between the serum sultopride concentration determined by radioimmunoassay and that determined by HPLC ($y=0.961x+0.008$, $r=0.995$).

Keywords—sultopride; serum; saliva; sulpiride; HPLC; radioimmunoassay

Sultopride is a neuroleptic drug belonging to the substituted benzamide family, some members of which (such as sulpiride) are already available for therapeutic use (Fig. 1). As



sultopride : $\text{R} = \text{SO}_2\text{C}_2\text{H}_5$
sulpiride : $\text{R} = \text{SO}_2\text{NH}_2$

Fig. 1. Chemical Structures of Sultopride and Sulpiride

methods for the measurement of sultopride concentration in body fluids, spectrofluorometry^{1,2)} and high-performance liquid chromatography (HPLC)³⁾ have been reported. However, the former requires 2 ml sample volume and the latter, 50 ml of urine. Furthermore, the use of radioimmunoassay is strictly regulated in Japan.

This report describes a HPLC method using a small sample for determination of sultopride in serum and saliva. The serum sultopride concentration determined by HPLC was compared with that determined by radioimmunoassay.⁴⁾

Experimental

Materials—Sultopride and sulpiride were supplied by Mitsui Pharmaceuticals Inc., and the internal standard, phenylethylmalonamide (PEMA), was supplied by Ayerst Co., Ltd. Other chemicals were of reagent grade. Blank serum was obtained from drug-free healthy volunteers. Serum containing sultopride was obtained from healthy volunteers after oral administration of sultopride.

Apparatus and Conditions—A liquid chromatograph, Shimadzu LC-2, equipped with a Shimadzu SPD-1 spectrophotometer was used. The chromatograph was fitted with a Nucleosil® 7C₁₈ (Macherey-Nagel Co., Ltd., 7.5 μm) column (4 mm \times 25 cm).

The mobile phase was prepared by mixing 17 parts of acetonitrile with 83 parts of 0.05 M phosphate buffer (pH 6.0, containing potassium chloride 25.35 g in 1000 ml) and degassed *in vacuo*. The flow rate of the mobile phase was

1.0 ml/min, and the column temperature was 30 °C. The spectrophotometer was set at 213 nm at 0.08 a.u.f.s.

Procedure—To 0.5 ml serum or saliva placed in a 20 ml test tube was added 1 ml of 1 M glycine-NaCl/1 N NaOH buffer (pH 10.0) and 10 ml of 0.05 µl/ml PEMA/methylene chloride. The mixture was shaken for 10 min and centrifuged for 5 min at 3000 rpm. The organic phase was transferred to another test tube and evaporated to dryness in a rotary vacuum evaporator. The residue was dissolved in 100 µl of the mobile phase. This solution was transferred to a microtube and centrifuged for 5 min at 7000 rpm. Twenty µl of this supernatant was injected into the liquid chromatograph. The peak height ratio of sultopride to internal standard was used for the quantitation of sultopride.

Results and Discussion

A chromatogram of drug-free serum or saliva to which sultopride had been added is shown in Fig. 2. Retention times of the internal standard (PEMA) and sultopride were 6.0 and 8.8 min, respectively.

The amount of the metabolite of sultopride, 2-oxo-sultopride, was thought to be negligible in serum and saliva, because 90% of the dose was excreted as unchanged sultopride and only a little as the metabolite in urine.²⁾ Thus, it is considered that the metabolite of sultopride in serum or saliva will not interfere with the assay of sultopride, even if the metabolite exists in plasma or saliva and the peak of metabolite overlaps that of sultopride.

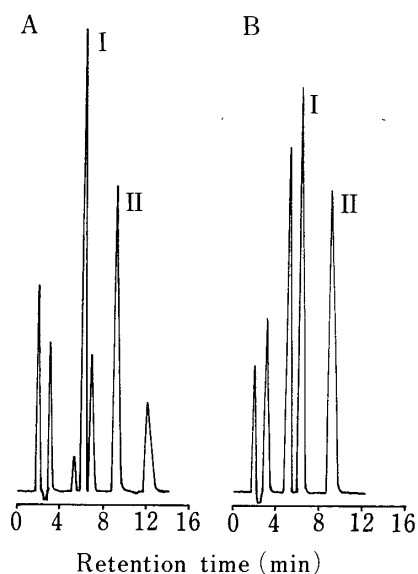


Fig. 2. Chromatograms of Drug-Free Serum and Saliva to Which Sultopride Had Been Added

A: serum, B: saliva. I: phenylethylmalonamide (internal standard). II: sultopride.

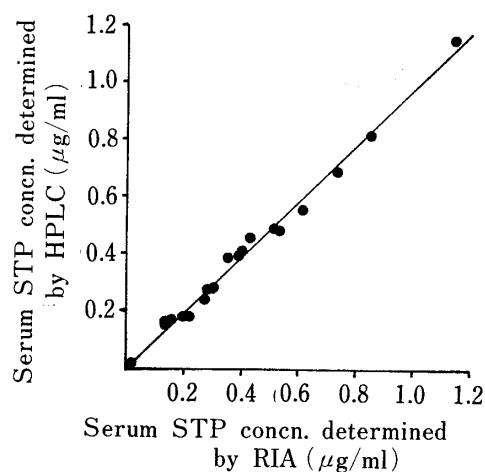


Fig. 3. Comparison of Serum Sultopride (STP) Concentrations Determined by Radioimmunoassay (RIA) and by High-Performance Liquid Chromatography (HPLC)

$$y = 0.961x + 0.008, r = 0.995, n = 19.$$

TABLE I. Within-Run Precision of Serum Sultopride Concentration Determined by High-Performance Liquid Chromatography

| Specimen No. | Serum concentration | | C.V. (%) | n |
|--------------|---------------------|-------|----------|---|
| | Mean | S.D. | | |
| | (µg/ml) | | | |
| 1 | 0.477 | 0.013 | 2.7 | 6 |
| 2 | 0.962 | 0.037 | 3.8 | 6 |
| 3 | 2.054 | 0.065 | 3.1 | 6 |

Since both sultopride and sulpiride are used in the treatment of psychiatric disorders, the possibility of the simultaneous determination of both drugs was investigated. The simultaneous determination of both drugs was found to be possible, since the retention time of sulpiride under the present HPLC conditions is 4 min.

The working curves for serum and saliva were linear from the limit of detection (*ca.* 0.04 $\mu\text{g/ml}$) to at least 4 $\mu\text{g/ml}$. The recoveries of sultopride added to serum and saliva (2 $\mu\text{g/ml}$) were $99.6 \pm 3\%$ and $99.8 \pm 3\%$ (mean \pm S.D., $n = 5$), respectively. The within-run precision in assay of sultopride added to serum is shown in Table I.

It was reported that the maximum serum sultopride concentration after single oral administration of 100 mg of the drug was about 0.8 $\mu\text{g/ml}$ in volunteers.²⁾ The saliva sultopride concentration was higher than the serum concentration, and the saliva/serum concentration ratio was 2 to 3.²⁾ The present HPLC method for determination of sultopride requires only 0.5 ml of serum or saliva.

The serum concentration of sultopride after single oral administration in normal volunteers as determined by radioimmunoassay⁴⁾ was in good agreement with that determined by HPLC (Fig. 3). Consequently, the present HPLC method is effective for the determination of sultopride in serum and saliva.

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