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## Note

# Determination of osalmid in plasma by high-performance liquid chromatography

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Osalmid, 4'-hydroxysalicylanilide, [1] is currently used clinically as a choleretic drug [2]. In the course of investigations on its absorption, distribution, metabolism, and excretion, a rapid and sensitive method of assay was required. High-performance liquid chromatography (HPLC) was found to meet the requirement.

The present paper describes the HPLC determination of osalmid in the plasma. The dog and human plasmas were assayed by the same procedure. The method can be applied to other body fluids.

## EXPERIMENTAL

### *Materials*

Osalmid was the product of Yoshitomi Pharmaceutical Ind., Fukuoka, Japan. 2,7-Dihydroxynaphthalene was the product of Wako, Osaka, Japan. Isopropyl ether was distilled immediately before use in the extraction procedure. All solvents and chemicals were of reagent grade.

### *Instrumentation*

An Hitachi Model 638 liquid chromatograph equipped with a universal in-

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jector and an Hitachi variable-wavelength ultraviolet effluent monitor operated at 300 nm was used. The column was a LiChrosorb Si-100 (Merck, Darmstadt, G.F.R.; particle size, 5 $\mu$m; 150 × 4 mm I.D.). The temperature of the column was maintained at 20°C. The flow-rate of the mobile phase was 0.8 ml/min.

*Extraction procedure*

Plasma (2.0 ml), water (1.0 ml), and phosphate buffer (pH 7.0; 15.0 ml) were mixed. Isopropyl ether containing 5% isoamyl alcohol (25.0 ml) was added to the mixture and the sample was extracted for 10 min with shaking. The organic layer (20.0 ml) was separated by centrifugation (ca. 1000 *g*, 10 min) and shaken for 5 min with 0.4 *M* NaOH (5.0 ml). After most part of the organic layer was discarded by aspiration, the residual solution was transferred to a glass-stoppered test tube. The organic layer was completely removed by centrifugation (ca. 1000 *g*, 5 min). To the aqueous layer (4.5 ml) were added 15% HCl (1.0 ml) and 10% isoamyl alcohol—*n*-butyl acetate (0.1 ml) containing 2,7-dihydroxynaphthalene (15 $\mu$g/ml) as a reference standard. The mixture was shaken for 5 min and then allowed to stand for 5 min in a water-bath (19°C). After most part of the aqueous layer was removed by centrifugation (ca. 1000 *g*, 5 min), the mixture was again allowed to stand for 5 min at 19°C. After the complete removal of the aqueous layer by centrifugation (ca. 1000 *g*, 2 min), a 10-$\mu$l volume of the organic layer was injected into the chromatograph.

For the preparation of the calibration curve, a mixture of drug-free plasma (2.0 ml), aqueous solution of osalmid (1.0 ml; 300—900 ng/ml), and phosphate buffer (15.0 ml) was carried through the procedure described above.

## RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of an extract of drug-free plasma and that of plasma containing osalmid (581 ng per 2 ml of plasma). The mobile phase was dichloromethane—*n*-hexane—methanol—5% acetic acid (66 : 30 : 4 : 0.35, v/v). The retention times were 5.4 min for osalmid and 6.9 min for the reference standard. The solvent system was found most suitable for the present purpose.

Increasing the concentration of acetic acid in the mobile phase made the retention times longer. When the proportion of 5% acetic acid was 0.30, the peaks of osalmid and the reference standard partially overlapped. Without the added acid, the osalmid peak was extremely broad because of its adsorption on the column.

With a solvent of a higher dichloromethane—*n*-hexane ratio, both peaks became closer, and with a solvent of lower dichloromethane concentration the peaks shifted to longer retention times. Increased methanol concentration in the solvent caused the peaks to overlap.

Using the most suitable solvent system described above, the ratio of the peak height of osalmid to that of the reference standard was plotted against the amounts of osalmid added to the standard. The calibration curves thus obtained passed through the origin and were linear up to at least 900 ng.

When the reference standard was added to plasma sample just before the

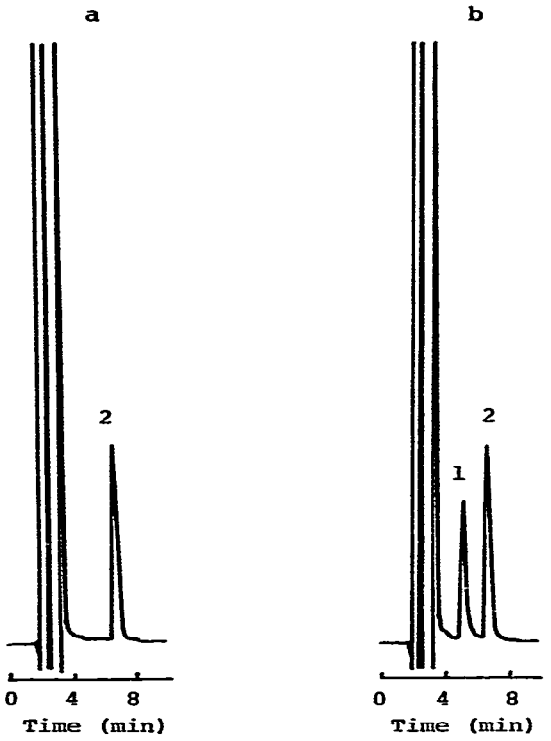


**Fig. 1. Chromatograms of (a) an extract of drug-free plasma of a dog and (b) that of plasma containing osalmid (581 ng per 2 ml). Peaks: 1 = osalmid; 2 = 2,7-dihydroxynaphthalene (internal standard).**

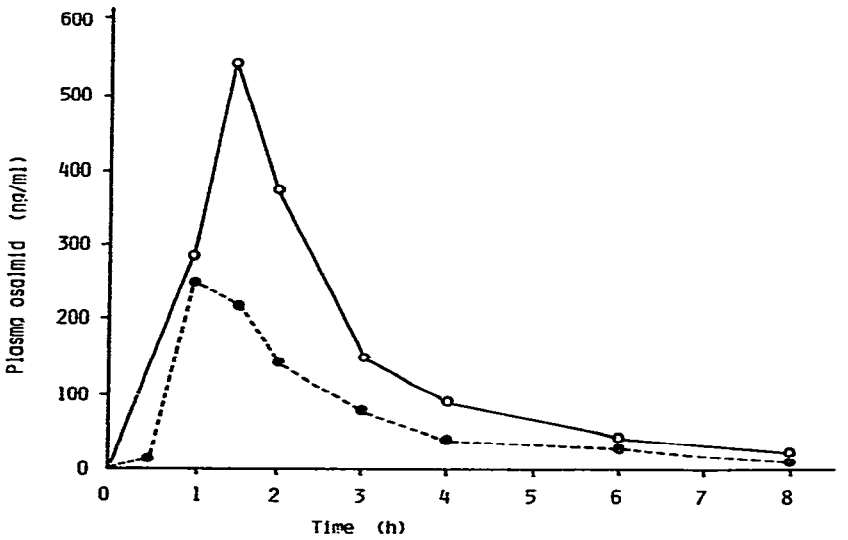


**Fig. 2. Plasma concentration of osalmid at various times after oral administration to dogs. Doses of osalmid per animal were 500 mg (closed circle) and 1000 mg (open circle).**

extraction, only 62% of the added amount was extracted and the results were sometimes irregular.

The precision was examined using plasma containing 212, 318, and 624 ng/ml ($n$=10). The coefficients of variation did not exceed 2.0%. The recovery of osalmid was checked by adding known amounts of osalmid (204, 301, 408, and 602 ng) to 1.0 ml of plasma ($n$=10). Recoveries were 98 ± 3% (mean ± S.D.). The method permits the accurate determination of osalmid in plasma at concentrations as low as 9 ng/ml.

Fig. 2 shows examples of the time—concentration curves in the plasma of dogs administered osalmid orally. The osalmid concentration in plasma increased immediately after the administration, reached a maximum in 1—1.5 h, and then decreased at a first-order rate. The biological half-life was about 1.5 h. Treatment of the plasma with 2 $M$ NaOH or β-glucuronidase prior to the assay procedure resulted in the enhancement of the analytical values. This indicates that a considerable amount of osalmid is present in conjugated forms. In the plasma of dogs administered osalmid intravenously, the conjugated forms were hardly present.

Details will be reported elsewhere of the results of studies of the bioavailability of various pharmaceutical preparations containing osalmid.

## REFERENCES

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