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# Determination of Osalmid in Plasma by High-Performance Liquid Chromatography*

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Osalmid, 4′-hydroxysalicylanilide, is currently used as a choleretic drug. A rapid and sensitive method of assay for osalmid was required in the course of investigations on its absorption, distribution, metabolism, and excretion. High-performance liquid chromatography (HPLC) was found to meet the requirement.

Plasma mixed with phosphate buffer was extracted by isopropyl ether containing 5% isoamyl alcohol. The organic layer was separated and shaken with aqueous NaOH. The aqueous layer was separated, acidified with aqueous HCl, and extracted with 10% isoamyl alcohol-n-butyl acetate containing 2,7-dihydroxynaphthalene as a reference standard. After the complete removal of the aqueous layer, a portion of the organic layer was injected into the chromatographic system.

A Hitachi liquid chromatograph system equipped with UV monitor operated at 300 nm and a LiChrosorb Si-100 column were used. The mobile phase was dichloromethane-n-hexane- methanol- 5% acetic acid (66 : 30 : 4 : 0.35, v/v). The ratio of the peak height of osalmid to that of the reference standard was plotted against the amount of osalmid added to the drug-free plasma. The calibration curve thus obtained was linear and passed through the origin.

The method permits the accurate determination of osalmid in plasma at concentrations as low as 9 ng/ml. The precision and recoveries were quite satisfactory. By the present method, plasma concentrations of osalmid at various times after oral administration to dogs were investigated.

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