Supplementary Material, Text S1

Benzisothiazolinone: Pharmacokinetics, Tissue Distribution, and Mass Balance studies in Rats

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Determination of the unbound fraction of BIT in rat plasma

Materials & Method

The plasma protein bindings were performed using a rapid equilibrium dialysis device and cellulose membranes with a molecular weight cutoff of 8000 (Thermo Scientific, Rockford, IL, USA) [1]. The rat plasma samples containing BIT at 5 and 50 μM, respectively, were dialyzed against a dialysis buffer, phosphate-buffered saline (PBS, 400 μL). The loaded dialysis plate was covered with sealing tape, placed on an orbital shaker at approximately 200 rpm, and incubated at 37 °C for 4 h. Thereafter, samples (100 μL) from both PBS and plasma chambers were collected and mixed with an equal volume of blank plasma and PBS, respectively. All samples were stored at −80 °C until LC-MS/MS analysis. The unbound fraction of BIT in rat plasma was calculated by dividing the BIT concentration in PBS by that in plasma.

Result

BIT was extensively bound to rat plasma proteins. The free fractions (%) of BIT at 5 and 50 μM in rat plasma were 0.562 ± 0.0152% and 0.606 ± 0.0416%, respectively, (n = 3, respectively).

[1] Cho D.Y., Bae S.H., Lee J.K., Kim Y.W., Kim B.T., Bae S.K. Selective inhibition of cytochrome P450 2D6 by Sarpogrelate and its active metabolite, M-1, in human liver microsomes. Drug Metab. Dispos. 2014;42:33–39. doi: 10.1124/dmd.113.054296.