INTRODUCTION

Curcumin is an orange-colored compound present in the Indian spice known as Turmeric. Although Curcumin has been claimed to have medicinal value for generations, recent studies have documented it as having anti-tumor activity when combined with other agents in a murine xenograph model.

To support the IND enabling tox studies for the proposed drug cocktail, LC-MS/MS assays were developed for each of the analytes for pharmacokinetic assessment.

Due to the instability of one of the analytes in the cocktail, whole blood assays were essential for assay development.

STUDY DESIGN

For the Tox Group - Male and female dogs (3/sex/group; four groups) were given either vehicle (Peptamen®) or the Curcumin cocktail (75, 100 or 125 mg/Kg/day) formulated with Peptamen® once daily by oral gavage for a minimum of 28 days.

For the Recovery Group: Male and female dogs (2/sex/group; control and high dose group) were given vehicle or the Curcumin cocktail (125 mg/Kg/day) formulated in Peptamen® once daily by oral gavage for 28 days, followed by a recovery of period of at least 28 days.

BIOANALYTICAL ANALYSES

Whole blood (1 mL) at each collection interval were rapidly transferred to tubes containing ice-cold acetonitrile (2 mL, 1:2, v/v). The tubes were capped, agitated for 20 min at 4 °C and frozen at -70 °C. Prior to analysis, samples were thawed in an ice-water bath and 2 mL of acetone was added to each tube. The samples were vortexed and agitated for 20 min at room temperature and then centrifuged. The supernatant was removed and the resulting supernatant (150 mcL) was combined with internal standard and then derivatized. Samples were then dried and reconstituted with methanol/acetone buffer mix.

The LC-MS/MS analyses were performed on an AB Sciex 5000 mass spectrometer in MRM positive mode, coupled with a Shimadzu UHPLC and Betasil C8 column (50 x 2.1 mm, 5 m).

RESULTS

The method was fully validated over a range of 2.00 to 100 ng/mL, in compliance with FDA bioanalytical method validation guidelines. Chromatograms of blank whole blood and LLOQ are presented in Figure 2. Curcumin was determined as stable in the processed whole blood samples at -70 °C for up to 35 days. Precision of the method ranges from 1.4 to 8.4%. Accuracy of the method ranges from -9.5 to 10.7%.

Additional xenograph investigations were then conducted for the drug cocktail with and without Curcumin in mice. Curcumin was confirmed as essential for anti-tumor activity observed for the oral product, although it was not detected.

Since Curcumin was not detectable in any of the species tested, supplemental methods were established to include two possible metabolites.

Samples from the murine xenograph investigations were able to confirm the presence of both conjugates following oral gavage (100 mg/Kg) in blood up to 19.7 ng/mL for the sulfate and 109 ng/mL for the glucuronide, with no detectable levels of Curcumin.

Fully validated methods have now been established in human whole blood for Curcumin, both of its conjugates, along with the other test compounds to support upcoming clinical trials.

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