Development and Validation of a RP-HPLC Method for Simultaneous Determination of Warfarin Enantiomers, Aspirin and Salicylic Acid in Beagle Plasma: Application to Pharmacokinetic Study

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SUMMARY. A novel RP-HPLC method for the simultaneous determination of R-warfarin (R-WAR), S-warfarin (S-WAR), aspirin (ASA) and its metabolite salicylic acid (SA) in beagle plasma was developed. Chromatographic separation was performed by a lux® cellulose-3 chiral column (250 mm × 4.6 mm, 5 μm) equipped with a UV detector (288 nm), using the isocratic elution of 0.5‰ formic acid in methanol and isopropanol (90/10, v/v) at a flow-rate of 1.0 mL/min. Calibration plots were linear over range of 0.029-7.5 μg/mL for (±)-WAR, 0.078-20 μg/mL for ASA, and 0.195-100 μg/mL for SA in beagle plasma. The validated method is successfully applied to a pharmacokinetic interaction study of WAR and ASA in beagles. The results show that coadministration of WAR with ASA may cause important pharmacokinetic interactions, which indicate that when WAR and ASA are administered together in clinical practice. Clinicians should pay more attention to bleeding risk and other safety-related issues.

KEY WORDS: Aspirin, HPLC, Pharmacokinetic interaction, Salicylic acid, Warfarin enantiomers.

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