**Bioanalytical method validation, biopharmaceutical and pharmacokinetic evaluation of GSK-650394, a serum- and glucocorticoid-regulated kinase 1 inhibitor**

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**Supplementary materials**

**Table S1.** LC–MS/MS working parameters

|  |  |  |
| --- | --- | --- |
| **Source parameters** | **GSK-650394** | **IS** |
| Declustering potential (V) | 196 | 66 |
| Entrance potential (V) | 10 | 10 |
| Collision energy (V) | 47 | 35 |
| Collision cell exit potential (V) | 22 | 24 |
| Ionspray voltage (V) | 5500 | 5500 |
| Temperature of ion source (°C) | 500 | 500 |
| Nebulizing gas (GS1) (psi) | 50 | 50 |
| Drying gas (GS2) (psi)  | 50 | 50 |
| Curtain gas (psi) | 30 | 30 |
| Collision gas (psi) | 9 | 9 |

**Method S1. Preparation of simulated gastrointestinal solutions**

The simulated gastrointestinal solutions were made by using biorelevant powder, following the instructions of the producer. For blank fasted state simulated gastric buffer (FaSSGF), 2.0 g of sodium chloride (NaCl) was dissolved in 900 mL of filtrated water, and after modifying pH to 1.6 with hydrochloric acid (HCl), adding water to 1000 mL. For blank fasted state simulated intestinal buffer (FaSSIF), 0.105 g of sodium hydroxide (NaOH) pellets, 0.8510 g of monobasic sodium phosphate anhydrous (NaH2PO4), and 1.547 g of NaCl were dissolved in 230 mL of water, and after modifying the pH to 6.5 with NaOH or HCl, adding water to 250 mL. For blank fed state simulated intestinal buffer (FeSSIF), 0.202 g of NaOH pellets, 0.433 g of glacial acetic acid (CH3COOH), and 0.594 g of NaCl were dissolved in 45 mL of water, modifying pH to 5.0 with NaOH or HCl, adding water to a volume of 50 mL. Finally, FaSSGF, FaSSIF and FeSSIF solution were freshly made on the day of studies by dispensing biorelevant powder (Biorelevant, London, United Kingdom) into the prepared blank fluids. FaSSGF solution was made by adding 0.0597 g of biorelevant powder to 1000 mL of blank FaSSGF buffer. FaSSIF solution was made by adding 0.5600 g of biorelevant powder to 250 mL of blank FaSSIF buffer. FeSSIF solution was prepared by adding 0.5510 g of biorelevant powder to 50 mL of blank FeSSIF buffer. The components of each simulated fluid were summarized in table S2.

**Table S2.** Components of simulated gastrointestinal solutions

|  |  |  |  |
| --- | --- | --- | --- |
| **Components** | **FaSSGF** | **FaSSIF** | **FeSSIF** |
| NaCl (g) | 1.999 | 1.547 | 0.594 |
| NaOH (g) |  | 0.105 | 0.202 |
| NaH2PO4 (g) |  | 0.851 |  |
| CH3COOH (g) |  |  | 0.433 |
| pH adjustment to | 1.6 | 6.5 | 5 |
| Biorelevant powder (g) | 0.0597 | 0.56 | 0.551 |
| Total volume (L) | 1 | 0.25 | 0.05 |

**Table S3.** List of glucuronosyltransferase inhibitors used for UGT phenotyping study.

|  |  |  |  |
| --- | --- | --- | --- |
| **Substrate** | **UGT types** | **Inhibitor** | **Final concentration** |
| GSK-650394 | UGT 1A1 | Atazanavir  | 50 µM |
| UGT 1A3 | Celastrol  | 50 µM |
| UGT 1A4 | Hecogenin  | 10 µM |
| UGT 1A6 | Troglitazone  | 50 µM |
| UGT 1A9 | Niflumic acid  | 50 µM |
| UGT 2B7 | Mefenamic acid  | 50 µM and 200 µM |
| Non-selective | Quinidine  | 200 µM and 1 mM |
| Non-selective | Diclofenac | 200 µM |