**Tables**

**Table S1: Quantitative analysis data of GFB through various analytical methods**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Chromatographic technique**  | **Mobile phase**  | **Detector** | **Flow rate**  | **Concentration range (Calibration curve)** | **LOD**  | **LOQ** |  |
| LCMS/MS | Acetonitrile: water (70:30 v/v) | Triple-quadrupole  | 0.15 mL/min  | 1–1000 ng/mL (human plasma )5–1000 ng/mL for (mouse plasma) | - | 0.5 ng/mL | Zhao et., al 2005 |
| HPLC | Acetonitrile : Phosphate buffer (70:30 v/v) | Photodiode array  | 1 mL/min | 2-14 µg/mL | 0.469 µg/mL | 1.42 µg/mL | Vijetha and Reddy, 2020  |
| HPLC  | Acetonitrile: 40 mM ammonium formate buffer pH 2.5 (30:70 v/v) | U.V. | 1 mL/min | 0.2 to 12 µg/mL | 37.852 ng/mL | 114.702 ng/mL | Ks et al., 2017 |
| HPLC  | 130 mM ammonium acetate: acetonitrile, pH 5;(63:37, v/v)  | Photodiode array | 1 mL/min | 0.1 to 2 µg/mL | 0.012 –0.033 µg/mL | 0.04–0.10 µg/mL | Chandrashekara et al., 2014 |
| Rapid resolution liquid chromatography (RRLC) | Ammonium acetate : acetonitrile (60:40 v/v/) | Variable wavelength detector (VWD) | 0.5 mL/min | 50% to 150%  | 0.01% | 0.03% | Venkataramanna et., al 2011 |
| HPLC/LCMS | Acetonitrile : formic acid (75:25 v/v) | PDA and ELSD  | 500 μL/min |  2 to 10 ppm 4µg/mL | 5.56% | 11.12% | Kotte et.,al 2012 |
| HPLC/MS/MS | Formic acid : acetonitrile  | Quadrupole mass spectrometer | 0.4 mL/min | 1–200 ng/ml | - | 1 ng/mL | Zheng et., al 2016 |

**Table S2: Box Behnken Design used for optimization of mobile phase condition with their obtained responses**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Run** | **ACN Composition (%)** | **Flow rate (mL/min)** | **Ammonium acetate concentration (%)** | **Retention time** | **Area** | **Theoretical plates** | **Tailing factor** |
| 1. | 60 | 1.3 | 0.5 | 5.123±0.98 | 6702590±1212 | 6451.04±3.2 | 1.32±0.51 |
| 2. | 60 | 0.7 | 0.5 | 9.431±1.4 | 12301700±4176 | 8874±1.8 | 1.2±0.83 |
| 3. | 60 | 1.3 | 1.5 | 4.98±1.23 | 6986530±8712 | 7947±1.67 | 1.14±0.8 |
| 4. | 60 | 0.7 | 1.5 | 9.191±0.67 | 12916000±6184 | 11648±3.9 | 1.1±±0.26 |
| 5. | 80 | 1.3 | 1 | 3.441±0.98 | 6402250±1962 | 8283±4.1 | 1.2±0.51 |
| 6. | 40 | 0.7 | 1 | 30.513±0.56 | 12377600±6190 | 7552±1.6 | 1.31±0.95 |
| 7. | 40 | 1 | 0.5 | 22.802±0.65 | 8609840±9012 | 5920±1.5 | 1.55±0.15 |
| 8. | 40 | 1 | 1.5 | 21.049±0.23 | 8697940±3891 | 7084±2.6 | 1.35±0.61 |
| 9. | 40 | 1.3 | 1 | 16.817±0.51 | 6689970±1836 | 5541±3.1 | 1.41±0.87 |
| 10. | 80 | 1 | 0.5 | 4.484±0.74 | 8624680±3618 | 9459±3.9 | 1.41±0.91 |
| 11. | 80 | 0.7 | 1 | 6.342±0.39 | 12140800±9812 | 11186±2.7 | 1.2±0.43 |
| 12. | 80 | 1 | 1.5 | 4.457±0.18 | 8646920±4617 | 10492.7±2.9 | 1.31±0.67 |
| 13. | 60 | 1 | 1 | 6.556±1.1 | 8433100±9125 | 8160±1.3 | 1.15±0.17 |
| 14. | 60 | 1 | 1 | 6.557±1.61 | 8382800±7632 | 8292±1.6 | 1.12±0.38 |
| 15. | 60 | 1 | 1 | 6.561±0.82 | 8610970±1742 | 8450±2.9 | 1.13±0.69 |

**Table S3: System suitability data of analyte**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Levels** | **LLQC** | **LQC** | **MQC** | **HQC** |
| **Rep-1** | 423776 | 2129003 | 4236785 | 6354543 |
| **Rep-2** | 425790 | 2119103 | 4238123 | 6359654 |
| **Rep-3** | 423678 | 2129283 | 4236896 | 6351234 |
| **Rep-4** | 424690 | 2137893 | 4239871 | 6334566 |
| **Rep-5** | 423912 | 2126287 | 4231567 | 6349787 |
| **Rep-6** | 424992 | 2118047 | 4248290 | 6399544 |
| **Mean** | 424473 | 2126603 | 4237123 | 6358221 |
| **S.D.** | 834.6849 | 7347.945 | 5502.713 | 21921.14 |
| **R.S.D.** | 0.19664 | 0.345525 | 0.129869 | 0.344768 |

**Table S4: Robustness data of analyte**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters**  | **Variation**  | **%RSD (Mean peak area)** | **Theoretical plates** | **Tailing factor** | **Retention time**  |
| **Wavelength (nm)** | **250** | 0.654 | 8292±2.3 | 1.12±0.5 | 6.5±0.76 |
| **254** | 0.984 | 8356±3.2 | 1.14±0.12 | 6.51±0.54 |
| **258** | 0.234 | 8234±2.1 | 1.13±0.34 | 6.52±0.65 |
| **Column Temperature (℃)** | **20** | 0.345 | 8345±1.4 | 1.12±.32 | 6.57±0.27 |
| **25** | 0.754 | 8268±4.3 | 1.10±0.99 | 6.66±0.76 |
| **30** | 0.981 | 8324±1.2 | 1.11±0.67 | 6.65±0.12 |
| **Injection volume (µL)** | **15** | 0.576 | 8564±1.6 | 1.14±0.32 | 6.51±0.34 |
| **20** | 0.921 | 8126±0.98 | 1.13±0.21 | 6.58±0.88 |
| **25** | 0.213 | 8298±0.54 | 1.12±.54 | 6.69±0.91 |

**Table S5: Ruggedness data of analyte**

|  |  |  |  |
| --- | --- | --- | --- |
| **Theoretical concentration**  |  **% Recovery** |  |  |
| **HPLC-1** | **HPLC-2** | **Analyst-1** | **Analyst-2** |
| 5 | 99.8±14 | 100.1±2.1 | 99.45±1.3 | 99.72±0.76 |
| 25 | 99.9±1.2 | 99.8±1.3 | 99.98±1.5 | 99.51±1.4 |
| 50 | 98.4±1.6 | 98.23±0.4 | 99.6±1.1 | 100.01±0.9 |
| 75 | 100.4±0.7 | 100.34±1.1 | 98.5±1.5 | 100.12±1.1 |

**Table S6: Stability data of analyte (n=3) for 30 days**

|  |  |  |  |
| --- | --- | --- | --- |
| **Level**  | **Freeze thaw cycle** | **Short term**  | **Long term (-20℃ for 2 weeks)** |
| **4h** | **12h** |
| 5 | 99.3±1.2 | 100.5±0.9 | 100.2±1.2 | 100.2±1.4 |
| 25 | 99.1±2.5 | 100.2±2.1 | 98.8±4.5 | 99.1±4.4 |
| 50 | 100.7±1.9 | 98.3±5.3 | 99.4±2.2 | 99.4±0.8 |
| 75 | 99.1±2.3 | 99.3±33 | 99.9±3.1 | 98.7±2.9 |