**Supplementary information**

**Borneol and α-asarone as adjuvant agents for improving blood-brain barrier permeability of puerarin and tetramethylpyrazine by activating adenosine receptors**

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A



B



Figure S1. (A)The chromatogram map of mixed standard plasma sample. (B)The chromatogram map of mixed standard brain tissue homogenate sample. PB: Phenobarbital; STR: strychnine; TMP: Tetramethylphrazine; PUE: Puerarin



A

B



Figure S2. (A) The chromatogram map of blank plasma sample; (B) The chromatogram map of blank brain tissue homogenate sample.

Table S1. Linear regression equations, correlation coefficients and the limit of quantitation for PUE and TMP in plasma samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Linear range(ng mL-1) | Linear regression equations | correlation coefficients (R2) | limit of quantitation (ng mL-1) |
| PUE | 1.17 — 1000 | y = 0.0188x - 0.0167  | 0.9999 | 0.58 |
| TMP | 8.67 — 1833 | y = 0.0049x + 0.0871 | 0.9987 | 6.51 |

Table S2. Linear regression equations, correlation coefficients and the limit of quantitation for PUE and TMP in brain tissue homogenate samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Linear range(ng mL-1) | Linear regression equations | correlation coefficients (R2) | limit of quantitation (ng mL-1) |
| PUE | 1.17 — 1000 | y = 0.0101x - 0.0114 | 0.9999 | 0.45 |
| TMP | 8.67 — 1833 | y = 0.0087x + 0.0480 | 0.9998 | 5.33 |

Table S3. Matrix effects of PUE in plasma samples.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Low dose (2.5ng mL-1) | Median dose (66.7ng mL-1) | High dose (800ng mL-1) |
|  | A1 | A0 | A1 | A0 | A1 | A0 |
| Area | 3569 | 3800 | 156428 | 142529 | 1589854 | 1829686 |
| 3346 | 4069 | 153926 | 151997 | 1646856 | 1766822 |
| 2946 | 4057 | 151882 | 139639 | 1658973 | 1596833 |
| 3128 | 3370 | 161367 | 144377 | 1766364 | 1878782 |
| 3558 | 3823 | 152991 | 143933 | 1807060 | 1795511 |
| RSD (%) | 8.21 | 7.41 | 2.43 | 3.17 | 5.31 | 6.05 |
| Matrix effects (%) | 86.55 | 107.49 | 95.51 |

Table S4. Matrix effects of TMP in plasma samples.

|  |  |  |  |
| --- | --- | --- | --- |
| 　 | Low dose (16.7ng/mL) | Median dose (200ng/mL) | High dose (1500ng/mL) |
| 　 | A1 | A0 | A1 | A0 | A1 | A0 |
| Area | 21401 | 25074 | 297580 | 315496 | 2021970 | 1931426 |
| 25511 | 24846 | 298730 | 324566 | 1858499 | 2024699 |
| 26550 | 25467 | 283502 | 309271 | 1935335 | 1886077 |
| 28350 | 27391 | 297915 | 307196 | 1810692 | 1879648 |
| 24355 | 26631 | 313430 | 308591 | 1969334 | 2065324 |
| RSD (%) | 10.29 | 4.20 | 3.55 | 2.30 | 4.42 | 4.27 |
| Matrix effects (%) | 97.49 | 95.27 | 98.04 |

Table S5. Matrix effects of PUE in brain tissue homogenate samples.

|  |  |  |  |
| --- | --- | --- | --- |
| 　 | Low dose (2.5ng mL-1) | Median dose (66.7ng mL-1) | High dose (800ng mL-1) |
| 　 | A1 | A0 | A1 | A0 | A1 | A0 |
| Area | 1826 | 2256 | 82512 | 88479 | 826294 | 784904 |
| 2096 | 2159 | 76012 | 86151 | 723373 | 780847 |
| 1930 | 2521 | 79188 | 86415 | 719407 | 899115 |
| 1926 | 2265 | 82469 | 85741 | 791131 | 825247 |
| 2102 | 2312 | 90216 | 91256 | 746065 | 766638 |
| RSD (%) | 6.06  | 5.83  | 6.44  | 2.62  | 6.07  | 6.62  |
| Matrix effects (%) | 85.82  | 93.69  | 93.83  |

Table S6. Matrix effects of TMP in brain tissue homogenate samples.

|  |  |  |  |
| --- | --- | --- | --- |
| 　 | Low dose (16.7ng/mL) | Median dose (200ng/mL) | High dose (1500ng/mL) |
| 　 | A1 | A0 | A1 | A0 | A1 | A0 |
| Area | 16849 | 16616 | 194056 | 200590 | 2043952 | 1973054 |
| 16055 | 15113 | 195611 | 209741 | 1947170 | 2035847 |
| 15187 | 17420 | 193399 | 205508 | 1951649 | 1927725 |
| 16976 | 13961 | 201656 | 211731 | 2100273 | 1896512 |
| 16304 | 14394 | 208621 | 200936 | 2022783 | 2061531 |
| RSD (%) | 4.40  | 9.50  | 3.25  | 2.45  | 3.22  | 3.53  |
| Matrix effects (%) | 104.99  | 96.58  | 101.73  |

Table S7. Accuracy, intra-day and inter-day recovery of PUE in plasma samples.

|  |  |  |  |
| --- | --- | --- | --- |
| 　 | Low dose(2.5ng mL-1) | Median dose (66.7ng mL-1) | High dose(800ng mL-1) |
| 　 | Concentration (μg/ml) | Recovery (%) | Concentration (μg/ml) | Recovery (%) | Concentration (μg/ml) | Recovery (%) |
| Day 1 | 2.49  | 99.46  | 68.71  | 103.02  | 1096.76  | 109.68  |
| 2.47  | 98.72  | 68.27  | 102.36  | 1079.28  | 107.93  |
| 2.55  | 101.89  | 61.61  | 92.37  | 1063.29  | 106.33  |
| 2.48  | 99.29  | 58.51  | 87.72  | 977.74  | 97.77  |
| 2.34  | 93.50  | 62.66  | 93.95  | 1065.65  | 106.57  |
| Day 2 | 2.55  | 102.15  | 68.27  | 102.36  | 1060.05  | 106.00  |
| 2.72  | 108.75  | 61.61  | 92.37  | 1064.08  | 106.41  |
| 2.43  | 97.37  | 58.51  | 87.72  | 1073.99  | 107.40  |
| 2.13  | 85.15  | 62.66  | 93.95  | 1002.76  | 100.28  |
| 2.22  | 88.73  | 72.91  | 109.32  | 968.74  | 96.87  |
| Day 3 | 2.36  | 94.42  | 66.94  | 100.36  | 968.95  | 96.90  |
| 2.28  | 91.31  | 69.62  | 104.38  | 892.65  | 89.26  |
| 2.63  | 105.20  | 68.55  | 102.78  | 956.81  | 95.68  |
| 2.33  | 93.32  | 67.90  | 101.80  | 1001.05  | 100.11  |
| 2.66  | 106.31  | 68.12  | 102.13  | 1047.63  | 104.76  |
| RSDintra-day (%) | 3.13  | 6.91  | 4.36  |
| RSDinter-day (%) | 3.52  | 6.74  | 3.87  |

Table S8. Accuracy, intra-day and inter-day recovery of TMP in plasma samples.

|  |  |  |  |
| --- | --- | --- | --- |
| 　 | Low dose (16.7ng/mL) | Median dose (200ng/mL) | High dose (1500ng/mL) |
| 　 | Concentration (μg/ml) | Recovery (%) | Concentration (μg/ml) | Recovery (%) | Concentration (μg/ml) | Recovery (%) |
| Day 1 | 16.71  | 100.06  | 220.31  | 110.16  | 1489.21  | 99.28  |
| 16.93  | 101.38  | 201.42  | 100.71  | 1429.94  | 95.33  |
| 15.88  | 95.09  | 206.15  | 103.08  | 1592.24  | 106.15  |
| 14.78  | 88.50  | 195.84  | 97.92  | 1538.40  | 102.56  |
| 16.49  | 98.74  | 197.68  | 98.84  | 1682.14  | 112.14  |
| Day 2 | 16.31  | 97.66  | 205.54  | 102.77  | 1456.78  | 97.12  |
| 16.99  | 101.74  | 199.14  | 99.57  | 1435.20  | 95.68  |
| 17.31  | 103.65  | 203.23  | 101.62  | 1459.29  | 97.29  |
| 16.54  | 99.04  | 191.25  | 95.63  | 1589.14  | 105.94  |
| 17.34  | 103.83  | 195.42  | 97.71  | 1572.84  | 104.86  |
| Day 3 | 15.98  | 95.69  | 192.54  | 96.27  | 1529.27  | 101.95  |
| 16.91  | 101.26  | 198.09  | 99.05  | 1548.75  | 103.25  |
| 15.84  | 94.85  | 199.08  | 99.54  | 1548.19  | 103.21  |
| 16.59  | 99.34  | 209.17  | 104.59  | 1620.20  | 108.01  |
| 15.92  | 95.33  | 203.85  | 101.93  | 1438.20  | 95.88  |
| RSDintra-day (%) | 5.35  | 4.79  | 6.26  |
| RSDinter-day (%) | 5.08  | 2.28  | 6.65  |

Table S9. Accuracy, intra-day and inter-day recovery of PUE in brain tissue homogenate samples.

|  |  |  |  |
| --- | --- | --- | --- |
| 　 | Low dose(2.5ng mL-1) | Median dose (66.7ng mL-1) | High dose(800ng mL-1) |
| 　 | Concentration (μg/ml) | Recovery (%) | Concentration (μg/ml) | Recovery (%) | Concentration (μg/ml) | Recovery (%) |
| Day 1 | 2.59  | 103.54  | 60.83  | 91.21  | 829.95  | 99.60  |
| 2.46  | 98.27  | 69.48  | 104.17  | 814.83  | 97.78  |
| 2.59  | 103.49  | 74.21  | 111.27  | 853.93  | 102.48  |
| 2.69  | 107.61  | 58.23  | 87.31  | 839.93  | 100.80  |
| 2.42  | 96.94  | 66.50  | 99.70  | 807.19  | 96.87  |
| Day 2 | 2.73  | 109.35  | 69.23  | 103.80  | 815.39  | 97.85  |
| 2.46  | 98.36  | 63.39  | 95.04  | 814.93  | 97.80  |
| 2.44  | 97.41  | 55.50  | 83.20  | 845.35  | 101.45  |
| 2.55  | 102.12  | 69.49  | 104.19  | 835.24  | 100.23  |
| 2.82  | 112.94  | 70.34  | 105.46  | 839.48  | 100.74  |
| Day 3 | 2.58  | 103.17  | 81.49  | 122.18  | 831.29  | 99.76  |
| 2.14  | 85.70  | 72.50  | 108.69  | 815.48  | 97.86  |
| 2.10  | 83.95  | 69.49  | 104.19  | 825.23  | 99.03  |
| 2.46  | 98.32  | 59.29  | 88.89  | 856.95  | 102.84  |
| 2.48  | 99.18  | 64.23  | 96.30  | 855.04  | 102.61  |
| RSDintra-day (%) | 4.26  | 9.80  | 2.27  |
| RSDinter-day (%) | 5.33  | 8.72  | 2.39  |

Table S10. Accuracy, intra-day and inter-day recovery of TMP in brain tissue homogenate samples.

|  |  |  |  |
| --- | --- | --- | --- |
| 　 | Low dose (16.7ng/mL) | Median dose (200ng/mL) | High dose (1500ng/mL) |
| 　 | Concentration (μg/ml) | Recovery (%) | Concentration (μg/ml) | Recovery (%) | Concentration (μg/ml) | Recovery (%) |
| Day 1 | 15.60  | 93.44  | 184.59  | 92.29  | 1432.95  | 95.53  |
| 17.63  | 105.57  | 182.96  | 91.48  | 1643.20  | 109.55  |
| 16.29  | 97.57  | 194.46  | 97.23  | 1390.44  | 92.70  |
| 16.84  | 100.85  | 199.48  | 99.74  | 1523.13  | 101.54  |
| 15.90  | 95.23  | 205.18  | 102.59  | 1487.39  | 99.16  |
| Day 2 | 15.82  | 94.75  | 220.48  | 110.24  | 1429.39  | 95.29  |
| 16.53  | 99.01  | 217.29  | 108.65  | 1565.04  | 104.34  |
| 16.83  | 100.79  | 198.48  | 99.24  | 1590.34  | 106.02  |
| 17.65  | 105.72  | 204.41  | 102.20  | 1580.76  | 105.38  |
| 17.43  | 104.39  | 203.29  | 101.65  | 1589.02  | 105.93  |
| Day 3 | 16.85  | 100.92  | 185.93  | 92.96  | 1459.49  | 97.30  |
| 15.36  | 91.96  | 204.29  | 102.15  | 1529.29  | 101.95  |
| 17.88  | 107.05  | 192.40  | 96.20  | 1603.02  | 106.87  |
| 17.99  | 107.71  | 198.23  | 99.12  | 1521.25  | 101.42  |
| 15.22  | 91.16  | 201.43  | 100.72  | 1486.39  | 99.09  |
| RSDintra-day (%) | 4.89  | 4.93  | 6.48  |
| RSDinter-day (%) | 4.55  | 6.90  | 6.53  |