Pharmacokinetic assay of sbk002 tablets and clopidogrel sulfate tablets in Beagle dogs’ plasma sample sbk002 test analysis report

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# Introduction

sbk002 is mainly the pharmacologically active metabolite of clopidogrel bisulfate. This assay used Beagle dogs as laboratory animals. 8 Beagle dogs were divided into two groups and orally administered sbk002 tablets and clopidogrel bisulfate tablets, collecting PK blood samples; after a 14-day washout period, cross-dosed and collected blood samples. Analyzed the concentration of sbk002 in PK blood samples and calculated the pharmacokinetic parameters of sbk002 in animals in vivo, providing research data for subsequent non-clinical and clinical assays.

PK blood sample collection time: the first period and the second period pre-dose and at 5 min, 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, and 24 h post-dosing. This assay uses a validated analytical method [LC-MS/MS quantitative measurement of sbk002 in Beagle dog plasma (analytical method number: sbk002-BA01)] to test the pharmacokinetic plasma sample sbk002 concentration.

# Study Materials and Methods

* 1. Reference Standard

Name/Code: sbk002-D;

Characterization: off-white crystalline powder;

content: 99.6 %;

Molecular weight: 337.82;

batch number: 180704;

Expiry date: 2020-08-07;

Storage condition: 15 ~ 25 ℃, airtight, shaded, dry;

Supplier: Chengdu Shipacon Biotechnology Co., Ltd.;

Manufacturer: Chengdu Shipacon Biotechnology Co., Ltd.;

Protection measures: Use the test article according to the "Occupational Health and Protection Manual," including wearing protective clothing, masks, and gloves;

Remaining Reference Standard disposal: Return to the sponsor.

* 1. internal standard

Name/Code: Tolbutamide;

Characterization: white powder;

Molecular weight: 270.35;

content: 99.8 %;

batch number: SLBR5486V;

Expiry date: 2019-06-08;

Storage condition: Shaded, room temperature, airtight;

Manufacturer: Sigma-Aldrich;

Protection measures: Use the test article according to the "Occupational Health and Protection Manual," including wearing protective clothing, masks, and gloves.

* 1. Major Reagents

| Reagent Name | Reagent Grade | Product Number | Manufacturer | batch number |
| --- | --- | --- | --- | --- |
| sterilized water for injection | / | / | Sichuan Kelun Pharmaceutical Co., Ltd. | M18080905-3 |
| Methanol | HPLC | A452 | Fisher Scientific | 185366 |
| Acetonitrile | HPLC | A998 | Fisher Scientific | 184266 |
| 2-Propanol | HPLC | A451 | Fisher Scientific | 179266 |
| Formic acid | LC/MS | A117 | Fisher Scientific | 182088 |
| dimethyl sulfoxide | HPLC | D159 | Fisher Scientific | 173815TF |
| 0.01M PBS | / | BL601A | biosharp | 183666 |
| TCEP | / | T107252 | Aladdin | D1803047 |

Note: TCEP stands for Tris(2-carboxyethyl)phosphine hydrochloride.

Biological matrix: Use EDTA-K2 anticoagulant and TCEP antioxidant Beagle dogs' blank plasma as biological matrix; self-made by Suzhou Huace Bio Technique Co., Ltd.; blood disposal conditions: centrifuged at 2 ~ 8 ℃, 4000 r/min for 10 min, separated upper plasma; plasma storage condition: stored at below -60 ℃.

* 1. Major Instruments

| name | Model | Manufacturer |
| --- | --- | --- |
| Liquid chromatography-mass spectrometry | ACQUITY UPLC I-Class + Xevo TQ-S | Waters |
| Chromatographic column | Kinetex® 1.7 μm C18 100 Å  100 × 2.1 mm | Phenomenex |
| analytical balance | XSE105DU | METTLER TOLEDO |
| Bench-top high-speed refrigerated centrifuge | 5810R/5804R | Eppendorf |
| sonicate cleaning machine | SB-5200DT | Ningbo Xinzhi Biotechnology Co., Ltd.  Co., Ltd. |
| 数显型1000 MP微孔板摇床 | ENFO-980179 | Talboys |
| Vortex mixer | LP Vortex Mixer | Thermo Scientific |

# ANALYSIS AND TEST

The ANALYSIS AND TEST method in this determination is validated by the "LC-MS/MS quantitative measurement of sbk002 in Beagle dog plasma methodology assay (study number: A2018030-BA01)."

# data acquisition and analyzed

all raw data within the facility collected manually or via data acquisition systems according to the study protocol and SOPs of Suzhou Huace Biological Technology Co., Ltd. Manually collected data were transcribed into Excel tables for analysis and reporting. Data collection and reporting electronic systems are as follows:

|  |  |  |
| --- | --- | --- |
| system | version | Use |
| Waters Unifi | 1.7.0.064 | LC-MS/MS testing concentration |

# result

Each analytical run calculates the concentration of Standard sample, QC sample, and unknown sample using the standard curve of that batch; the analytical run must meet the following acceptance criteria: System Suitability: the last 6 tests of sbk002 and the ratio of the peak area of the internal standard and the CV (Coefficient of variation, standard deviation/mean) of their retention time should be less than 10 %; Standard Curve: at least 75 % of calibration standards, including at least 6 effective concentrations, should meet the sample accuracy between 85 % ~ 115 % (lower limit of quantification accuracy should be between 80 % ~ 120 %); quality control: at least 2/3 of QC sample accuracy should be between 85 % ~ 115 %, and at least 1/2 of the QC sample accuracy at the same concentration should be between 85 % ~ 115 %. Internal standard: the CV of the peak area of the internal standard in the Standard Curve sample, QC sample, and to be tested sample within an analytical run should not exceed 20 %; Carry-over: carry-over effects should be tested in the analyzed process, minimized or avoided as much as possible. If unavoidable, note the sample arrangement order.

The assay sample analysis includes a total of 5 analytical runs, all result included in the final statistics, summarized in Appendix Tables 1.

The CV ratio of sbk002 to the peak area of the internal standard is between 0.72 % ~ 1.63 %; the CV of sbk002 retention time is between 0.00 % ~ 0.15 %; the CV of internal standard retention time is 0.00 %; System Suitability results are shown in Appendix Tables 2.

Within the linear range of the Standard Curve (0.5 ~ 500.0 ng/mL), accuracy is between 91.20 % ~ 107.27 % (the accuracy of the Lower Limit of Quantification is between 100.00 % ~ 106.00 %), regression coefficients (R2) are all greater than 0.99, Standard Curve results are shown in Appendix Tables 3 and Appendix Tables 4.

Quality Control Sample accuracy was between 92.67 % and 107.76 %, all QC sample accuracy should be between 85 % and 115 %, quality control results see Appendix Tables5 and Appendix Tables6.

CV of internal standard is between 2.75 % and 3.64 %, internal standard results see Appendix Tables7.

No Carry-over effects were seen in the analyzed process, carry-over results are shown in Appendix Tables 8.

The above results all meet the acceptance criteria of the analytical run.

The individual plasma concentration of the first and last dose of Beagle dogs is shown in Appendix Tables 9 and Appendix Tables 10.

Appendix Tables1 Beagle dogs orally administered sbk002 tablets and clopidogrel bisulfate tablets testing analytical run summary table

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Serial Number | analytical run | Date of Bench Top Process | Date of Injection | Verification content | Whether  Pass | Remark |
| 1 | A2018030-K01-01\_Run01 | 2018-12-19 | 2018-12-19 | D1 1M001、1M002、2M001、2M002  all samples (a total of 56 samples) | Yes | NA |
| 2 | A2018030-K01-01\_Run02 | 2018-12-20 | 2018-12-20 | D15 1M001、1M002、2M001、2M002  all samples (a total of 56 samples) | Yes | NA |
| 3 | A2018030-K01-01\_Run03 | 2018-12-21 | 2018-12-21 | D1 1M003、1M004、2M003、2M004  all samples (a total of 56 samples) | Yes | NA |
| 4 | A2018030-K01-01\_Run04 | 2018-12-23 | 2018-12-23 | D15 1M003、1M004、2M003、2M004  all samples (a total of 56 samples) | Yes | NA |
| 5 | A2018030-BA01\_Run08  (see A2018030-BA01) | 2019-01-01 | 2019-01-01 | D1 2M003 after dosing 24 h, D15 2M001  after dosing 24 h (a total of 2 samples) | Yes | The deviation between the second determination value and the first determination value is less than 20%, accepting the first determination result. |

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| --- | --- | --- | --- |
| Appendix Tables 2 System Suitability | | | |
| analytical run | sbk002 / peak area of internal standard CV | sbk002 retention time CV | internal standard retention time CV |
| A2018030-K01-01\_Run01 | 0.99% | 0.12% | 0.00% |
| A2018030-K01-01\_Run02 | 0.72% | 0.00% | 0.00% |
| A2018030-K01-01\_Run03 | 1.63% | 0.00% | 0.00% |
| A2018030-K01-01\_Run04 | 0.89% | 0.15% | 0.00% |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Appendix Tables3 Standard Curve sample concentration (ng/mL) | | | | | | | | | |
| theoretical concentration  analytical run | 0.5 | 1.0 | 5.0 | 10.0 | 50.0 | 100.0 | 400.0 | 500.0 | R2 |
| A2018030-K01-01\_Run01 | 0.50 | 1.00 | 4.96 | 9.88 | 50.70 | 97.51 | 398.73 | 501.37 | 0.9997 |
| 0.51 | 0.97 | 4.86 | 9.87 | 49.59 | 102.51 | 417.46 | 511.70 |
| A2018030-K01-01\_Run02 | 0.52 | 0.96 | 4.77 | 9.76 | 50.39 | 102.76 | 425.04 | 536.36 | 0.9983 |
| 0.50 | 0.96 | 4.74 | 9.46 | 48.87 | 102.94 | 398.84 | 512.61 |
| A2018030-K01-01\_Run03 | 0.53 | 0.94 | 4.79 | 10.01 | 50.91 | 102.36 | 418.79 | 522.19 | 0.9984 |
| 0.50 | 0.97 | 4.56 | 9.89 | 49.77 | 101.25 | 402.58 | 511.01 |
| A2018030-K01-01\_Run04 | 0.50 | 0.98 | 5.19 | 10.28 | 50.15 | 102.64 | 402.23 | 499.70 | 0.9997 |
| 0.50 | 1.00 | 4.97 | 9.97 | 49.43 | 100.14 | 376.40 | 497.18 |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Appendix Tables4 Standard Curve sample accuracy (%) | | | | | | | | |
| theoretical concentration (ng/mL)  analytical run | 0.5 | 1.0 | 5.0 | 10.0 | 50.0 | 100.0 | 400.0 | 500.0 |
| A2018030-K01-01\_Run01 | 100.00 | 100.00 | 99.20 | 98.80 | 101.40 | 97.51 | 99.68 | 100.27 |
| 102.00 | 97.00 | 97.20 | 98.70 | 99.18 | 102.51 | 104.37 | 102.34 |
| A2018030-K01-01\_Run02 | 104.00 | 96.00 | 95.40 | 97.60 | 100.78 | 102.76 | 106.26 | 107.27 |
| 100.00 | 96.00 | 94.80 | 94.60 | 97.74 | 102.94 | 99.71 | 102.52 |
| A2018030-K01-01\_Run03 | 106.00 | 94.00 | 95.80 | 100.10 | 101.82 | 102.36 | 104.70 | 104.44 |
| 100.00 | 97.00 | 91.20 | 98.90 | 99.54 | 101.25 | 100.65 | 102.20 |
| A2018030-K01-01\_Run04 | 100.00 | 98.00 | 103.80 | 102.80 | 100.30 | 102.64 | 100.56 | 99.94 |
| 100.00 | 100.00 | 99.40 | 99.70 | 98.86 | 100.14 | 94.10 | 99.44 |

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| Appendix Tables 5 Quality Control Sample concentration (ng/mL) | | | |
| theoretical concentration  analytical run | 1.5 | 25.0 | 375.0 |
| A2018030-K01-01\_Run01 | 1.52 | 26.07 | 401.23 |
| 1.46 | 26.29 | 397.49 |
| A2018030-K01-01\_Run02 | 1.52 | 26.45 | 402.71 |
| 1.48 | 26.55 | 404.10 |
| A2018030-K01-01\_Run03 | 1.42 | 24.16 | 376.04 |
| 1.46 | 23.39 | 371.07 |
| A2018030-K01-01\_Run04 | 1.45 | 23.79 | 374.81 |
| 1.39 | 23.75 | 359.83 |

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| --- | --- | --- | --- |
| Appendix Tables 6 Quality Control Sample accuracy (%) | | | |
| theoretical concentration (ng/mL)  analytical run | 1.5 | 25.0 | 375.0 |
| A2018030-K01-01\_Run01 | 101.33 | 104.28 | 106.99 |
| 97.33 | 105.16 | 106.00 |
| A2018030-K01-01\_Run02 | 101.33 | 105.80 | 107.39 |
| 98.67 | 106.20 | 107.76 |
| A2018030-K01-01\_Run03 | 94.67 | 96.64 | 100.28 |
| 97.33 | 93.56 | 98.95 |
| A2018030-K01-01\_Run04 | 96.67 | 95.16 | 99.95 |
| 92.67 | 95.00 | 95.95 |

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| --- | --- |
| Appendix Tables7 internal standard | |
| analytical run | internal standard CV |
| A2018030-K01-01\_Run01 | 3.55% |
| A2018030-K01-01\_Run02 | 2.75% |
| A2018030-K01-01\_Run03 | 3.05% |
| A2018030-K01-01\_Run04 | 3.64% |

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| Appendix Tables 8 Carry-over | | | |
| Date of Injection | sample | sbk002 Peak Area | peak area of internal standard |
| A2018030-K01-01\_Run02 | Carryover-1 | BLOQ | BLOQ |
| Carryover-2 | BLOQ | BLOQ |
| A2018030-K01-01\_Run03 | Carryover-1 | BLOQ | BLOQ |
| Carryover-2 | BLOQ | BLOQ |
| A2018030-K01-01\_Run04 | Carryover-1 | BLOQ | BLOQ |
| Carryover-2 | BLOQ | BLOQ |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Appendix Tables 9 Beagle dogs first (D1) administration of sbk002 tablets and clopidogrel sulfate tablets plasma sbk002 concentration (ng/mL) | | | | | | | | | | | | | | | |
| group | animal number | time point | | | | | | | | | | | | | |
| pre-dose | 5 min | 15 min | 30 min | 45 min | 1 h | 1.5 h | 2 h | 3 h | 4 h | 6 h | 8 h | 10 h | 24 h |
| sbk002 tablet group (30mg per dog) | 1M001 | BLOQ | BLOQ | 1.29 | 7.25 | 7.77 | 9.65 | 13.06 | 8.70 | 8.58 | 4.70 | 4.99 | 4.52 | 2.55 | 2.59 |
| 1M002 | BLOQ | 0.73 | 5.21 | 15.47 | 10.72 | 8.67 | 4.11 | 2.91 | 2.35 | 2.29 | 2.13 | 1.91 | 1.25 | 0.70 |
| 1M003 | BLOQ | 0.83 | 37.22 | 62.21 | 63.42 | 48.23 | 18.50 | 11.60 | 8.17 | 6.67 | 5.35 | 5.20 | 3.02 | 1.75 |
| 1M004 | BLOQ | 2.04 | 13.33 | 27.94 | 52.83 | 58.83 | 22.22 | 18.57 | 6.75 | 4.54 | 3.52 | 3.12 | 2.46 | 2.45 |
| Mean | 0.00 | 0.90 | 14.26 | 28.22 | 33.69 | 31.35 | 14.47 | 10.45 | 6.46 | 4.55 | 4.00 | 3.69 | 2.32 | 1.87 |
| SD | 0.00 | 0.85 | 16.11 | 24.21 | 28.58 | 25.98 | 7.87 | 6.51 | 2.85 | 1.79 | 1.48 | 1.47 | 0.75 | 0.86 |
| Clopidogrel sulfate tablet group (75 mg/each) | 2M001 | BLOQ | BLOQ | 1.38 | 4.92 | 21.83 | 57.34 | 32.03 | 20.62 | 7.43 | 5.61 | 4.24 | 3.18 | 2.99 | 0.90 |
| 2M002 | BLOQ | 0.64 | 4.15 | 10.50 | 26.19 | 39.29 | 36.58 | 26.10 | 13.18 | 8.39 | 5.96 | 3.81 | 3.02 | 0.92 |
| 2M003 | BLOQ | 0.71 | 174.52 | 87.44 | 50.88 | 26.26 | 17.92 | 10.84 | 7.35 | 6.09 | 5.19 | 3.86 | 3.61 | 13.38\* |
| 2M004 | BLOQ | 0.87 | 31.32 | 59.44 | 105.08 | 111.90 | 57.20 | 28.77 | 12.05 | 6.77 | 3.51 | 3.63 | 2.40 | 1.22 |
| Mean | 0.00 | 0.56 | 52.84 | 40.58 | 51.00 | 58.70 | 35.93 | 21.58 | 10.00 | 6.72 | 4.73 | 3.62 | 3.01 | 1.01 |
| SD | 0.00 | 0.38 | 82.24 | 39.70 | 38.26 | 37.69 | 16.25 | 7.92 | 3.05 | 1.21 | 1.07 | 0.31 | 0.49 | 0.18 |
| Note: BLOQ means below the lower limit of quantification; when calculating mean and SD, BLOQ is calculated as 0.00; \* means abnormal data, not included in statistical calculations. | | | | | | | | | | | | | | | |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Appendix Tables 10 Beagle dogs last (D15) administration of sbk002 tablets and clopidogrel sulfate tablets plasma sbk002 concentration (ng/mL) | | | | | | | | | | | | | | | |
| group | animal number | time point | | | | | | | | | | | | | |
| pre-dose | 5 min | 15 min | 30 min | 45 min | 1 h | 1.5 h | 2 h | 3 h | 4 h | 6 h | 8 h | 10 h | 24 h |
| Clopidogrel sulfate tablet group (75 mg/each) | 1M001 | BLOQ | 10.71 | 78.83 | 50.44 | 39.20 | 18.92 | 9.25 | 6.21 | 7.22 | 7.66 | 5.19 | 3.39 | 2.02 | 1.35 |
| 1M002 | BLOQ | BLOQ | BLOQ | 11.97 | 36.08 | 56.56 | 18.32 | 7.36 | 4.56 | 3.67 | 2.57 | 2.04 | 1.53 | 0.63 |
| 1M003 | BLOQ | 1.75 | 41.96 | 37.63 | 25.43 | 17.68 | 7.16 | 5.62 | 4.85 | 4.66 | 3.18 | 2.53 | 2.11 | 0.88 |
| 1M004 | BLOQ | 3.74 | 44.81 | 26.06 | 19.58 | 11.80 | 7.26 | 6.21 | 7.06 | 5.30 | 4.37 | 3.10 | 2.16 | 3.80 |
| Mean | 0.00 | 4.05 | 41.40 | 31.53 | 30.07 | 26.24 | 10.50 | 6.35 | 5.92 | 5.32 | 3.83 | 2.77 | 1.96 | 1.67 |
| SD | 0.00 | 4.70 | 32.28 | 16.40 | 9.15 | 20.45 | 5.30 | 0.73 | 1.41 | 1.70 | 1.18 | 0.60 | 0.29 | 1.45 |
| sbk002 tablet group (30mg per dog) | 2M001 | BLOQ | BLOQ | 2.27 | 14.69 | 35.12 | 19.85 | 12.12 | 13.11 | 7.57 | 5.48 | 8.81 | 4.32 | 3.40 | 16.48\* |
| 2M002 | BLOQ | BLOQ | 27.55 | 45.38 | 38.29 | 18.77 | 7.34 | 4.50 | 5.97 | 6.22 | 4.76 | 3.30 | 2.09 | 0.65 |
| 2M003 | BLOQ | BLOQ | 22.73 | 34.39 | 30.31 | 26.56 | 11.01 | 5.63 | 5.25 | 6.13 | 4.72 | 3.69 | 3.65 | 3.11 |
| 2M004 | BLOQ | BLOQ | 38.36 | 51.13 | 49.51 | 50.71 | 37.09 | 18.98 | 9.92 | 5.89 | 4.50 | 3.25 | 4.14 | 1.55 |
| Mean | 0.00 | 0.00 | 22.73 | 36.40 | 38.31 | 28.97 | 16.89 | 10.56 | 7.18 | 5.93 | 5.70 | 3.64 | 3.32 | 1.77 |
| SD | 0.00 | 0.00 | 15.12 | 16.05 | 8.16 | 14.90 | 13.62 | 6.79 | 2.07 | 0.33 | 2.08 | 0.49 | 0.88 | 1.24 |
| Note: BLOQ means below the lower limit of quantification; when calculating mean and SD, BLOQ is calculated as 0.00; \* means abnormal data, not included in statistical calculations. | | | | | | | | | | | | | | | |