Supplementary information.

**HPLC Conditions**

|  |  |  |
| --- | --- | --- |
| Column | : | Acquity BEH C18 1.7 m 30 x 4.6 mm (PN 186004643) |
| Gradient profile | : | Time (minutes)0.05.2 5.75.8 | Water  (A)850085 | Acetonitrile (B)590905 | 250 mM ammonia(D)10101010 |
| Stop time | : | 6.2 minutes |
| Post Run | : | 2.3 minutes |
| Flow rate | : | 2.0 mL/min |
| Detection | : | 210 nm |
| Injection volume | : | 5 L |
| Column temperature | : | 40C |

**Calculations**

*Initial loading calculation milligram scale*

Density of the feed solution measured as 0.92 g/ml

Therefore, 1000mL of this solution = 920 g

Solution assay for the product = 25% w/w

Therefore in 1 litre = 0.25 x 920 = 230 g of product

Molecular Mass of the product = 378.5

Molarity of solution = 230 / 378.5 = 0.60 M

Therefore, 1mL of solution contains 0.6/1000 x 378.5 g of product = 227 mg

**Milligram to multi-gram scale calculation**

H= Volume of feed solution 2 mL (containing 0.5 g of product).

I= Mass of SCX-2 ion-exchange stationary phase packed in a cartridge 10 g.

J= mass of SCX-2 ion-exchange stationary packed pack in a 25 mm id column 60 g.

(H/I) \*J= Volume to load on at the multi-gram scale in mL.

(2/10) \* 60 = 12 mL

**Multi-gram to multi-kilogram scaling calculation**

K= Feed volume loaded at the multi-gram scale

L= mass of SCX-2 ion-exchange stationary packed at multi-gram scale 60 g

M= mass of SCX-2 ion-exchange stationary packed multi-kilogram scale 21000 g

(K/L) \* M = Volume to load on at the multi-kilogram scale in mL

|  |  |  |
| --- | --- | --- |
| Batch  | Multi-gram scale Volume loaded mL (K) | Multi-kilogram scale maximum volume to load mL (M) |
| 1 | 9 | 3150 |
| 2 | 8 | 2800 |

Batch 1 volume was 18L, therefore the batch was sub-divided into 6 aliquots.

Batch 2 volume was 23.4L, therefore the batch was sub-divided into 9 aliquots.

Table A

Physical and chemical properties of the selected cation exchange resin.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Resin | Functional Group | Matrix | Bead Size μm | Capacity meq/L |  “Column Volume” / mL\* |
| ISOLUTE ® SCX-2 | Sulfonic acid | Silica | 50 | 0.4 | 20 |

A “column volume” (CV) was initially taken as the actual physical space occupied by the resin and associated pipework; the interstitial volume and pore volume were not taken into consideration during development

Table B: Experimental details and scaling

|  |  |
| --- | --- |
| Milligram Scale. | Multi-gram/Multi-kilogram Scale.  |
| Methanol HPLC Grade HiPerSolv CHROMANORM supplied by VWR International Leicestershire, UK.  | Methanol 100% synthetically manufactured, supplied by Alfa-Aesar Lancashire, UK. |
| Trifluoroacetic acid HPLC grade ≥99.0% supplied by Sigma-Aldrich. | Trifluoroacetic acid HPLC grade ≥99.0% supplied by Alfa-Aesar diluted to 2M with methanol.  |
| N/A | Acetic acid (glacial) *ReagentPlus*®, ≥99% supplied by Sigma-Aldrich. |
| N/A | Methanesulphonic Acid grade ≥99.0% supplied by Sigma-Aldrich. |
| AZ13757020 solution in Cyclopentylmethylether (CPME) supplied by AstraZeneca Pharmaceutical Sciences, Macclesfield, UK at varying concentrations. | AZ13757020 solution in Cyclopentylmethylether (CPME) supplied by AstraZeneca Pharmaceutical Sciences, Macclesfield, UK at varying concentrations. |
| N/A | Sodium hydroxide solution 10M supplied by Alfa-Aesar. |
| Methanolic ammonia 7M supplied by Sigma-Aldrich diluted to 2M with methanol as required. | Methanolic ammonia 7M supplied by Alfa-Aesar diluted to 2M with methanol. |