Electronic Supplementary Material (ESI) for Analyst.


# An optimised assay for quantitative, high-throughput analysis of polysialyltransferase activity

Sara M. Elkashef, Mark Sutherland, Laurence H. Patterson, Paul M. Loadman and Robert A. Falconer*

**Table 1.** HPLC gradient for DMB-DP3 purification using anion exchange chromatography (Flow rate 1 ml/min).

| Time (minutes) | % Mobile phase A | % Mobile phase B |
|---|---|---|
| **0** | 100 | 0 |
| **5** | 100 | 0 |
| **25** | 98 | 2 |
| **30** | 100 | 0 |

**Table 2.** HPLC gradient for analysis of polyST enzyme activity using anion exchange HPLC

| Time (minutes) | % Mobile phase A | % Mobile phase B |
|---|---|---|
| **0** | 100 | 0 |
| **2** | 100 | 0 |
| **9** | 63 | 37 |
| **11** | 100 | 0 |
| **12** | 100 | 0 |

**Table 3.** Conditions explored for DMB-DP3 purification by RP-HPLC.

| System | % Mobile phase A | % Mobile phase B | Method |
|---|---|---|---|
| **1** | 1% methanol, 0.01% formic acid (pH 3.5) | 55% methanol, 0.01% formic acid (pH 5.8) | Isocratic separation of 15% mobile phase B for 70 minutes |
| **2** | 5 mM ammonium formate buffer (pH 6) | 55% methanol, 0.01% formic acid (pH 5.8) | Isocratic separation of 15% mobile phase B for 70 minutes |
| **3** | 5 mM ammonium formate buffer (pH 6) | 55% methanol (pH 7.2) | Isocratic separation of 10% mobile phase B for 70 minutes |

**Table 4.** Gradient initially used for DMB-DP3 purification using anion exchange–HPLC system (flow rate 1.0 ml/min).

| Time (minutes) | % Mobile phase A | % Mobile phase B |
|---|---|---|
| **0** | 100 | 0 |
| **5** | 100 | 0 |
| **15** | 92 | 2 |
| **30** | 100 | 0 |