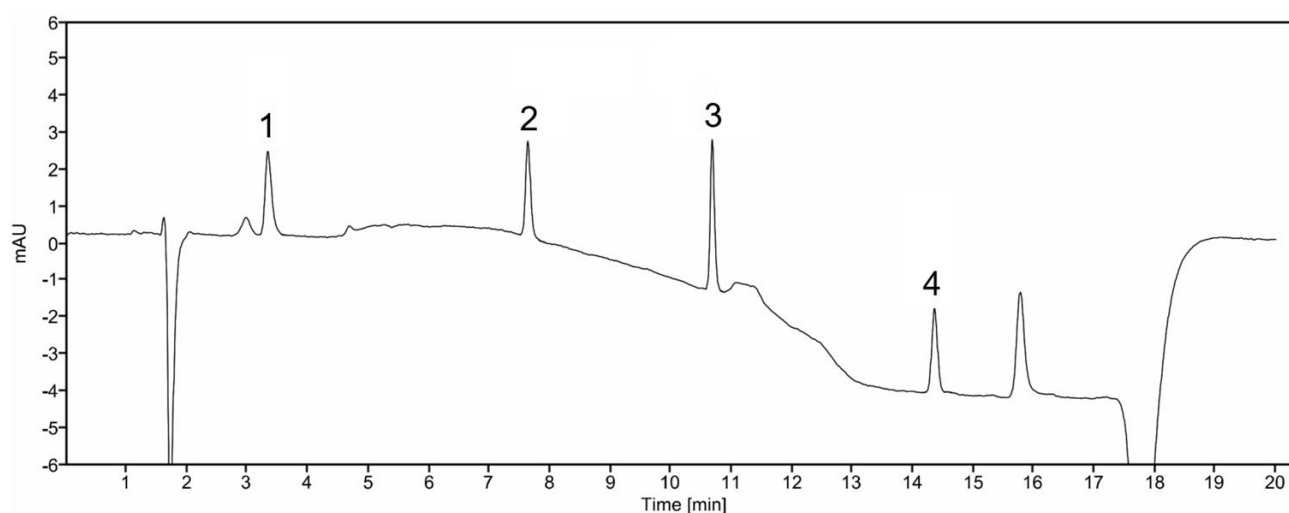




## Bumetanide Injection – BP 2027

These chromatograms are provided for information only as an aid to analysts and are intended as guidance for the interpretation and application of BP monographs.

Typical chromatogram for solution (3) from the Related Substances test for Bumetanide Injection as published in BP 2027.

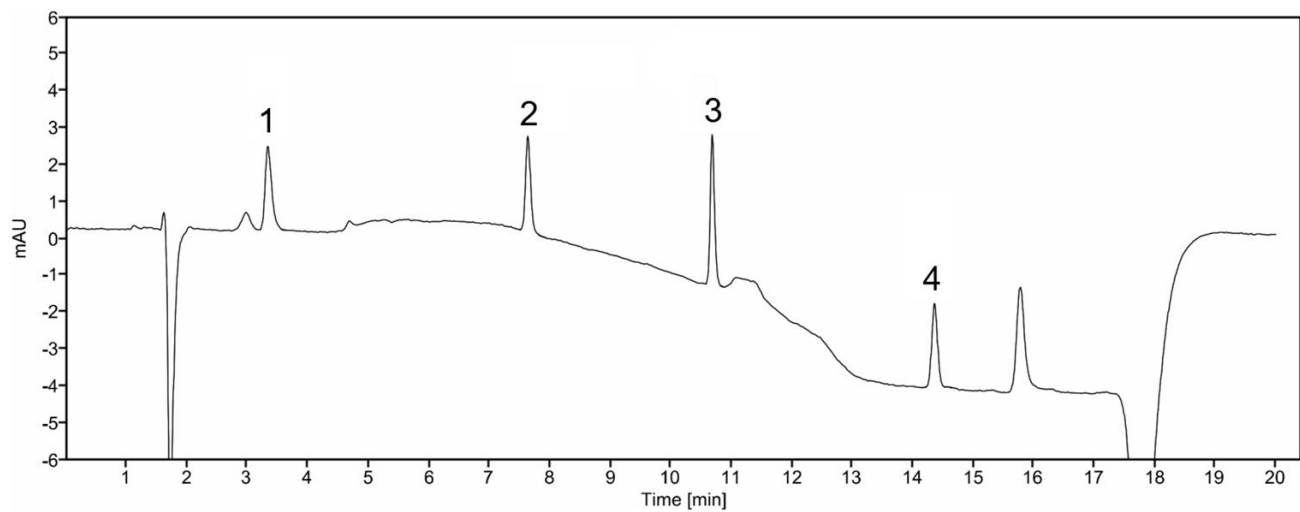


Peak ID: 1: Impurity B. 2: Impurity A. 3: Bumetanide. 4: Impurity C.

|                         |                                                                        |
|-------------------------|------------------------------------------------------------------------|
| <b>Column</b>           | XTerra C18 (150 mm x 4.6 mm, 3.5 µm)                                   |
| <b>Method Ref.</b>      | Related Substances for the Bumetanide Injection monograph from BP 2027 |
| <b>Mobile Phase A</b>   | 0.5% v/v formic acid                                                   |
| <b>Mobile Phase B</b>   | Methanol                                                               |
| <b>Diluent</b>          | 40% methanol                                                           |
| <b>Flow rate</b>        | 1.0 mL/min                                                             |
| <b>Column Temp</b>      | 30°C                                                                   |
| <b>Injection Volume</b> | 50 µL                                                                  |

|                |                           |                           |                  |
|----------------|---------------------------|---------------------------|------------------|
| Detection      | 254 nm                    |                           |                  |
| Gradient       |                           |                           |                  |
| Time (minutes) | Mobile phase A<br>(% v/v) | Mobile phase B<br>(% v/v) | Comment          |
| 0 – 2          | 60                        | 40                        | isocratic        |
| 2 – 10         | 60 → 20                   | 40 → 80                   | linear gradient  |
| 10 – 15        | 20                        | 80                        | isocratic        |
| 15 – 15.1      | 20 → 60                   | 80 → 40                   | linear gradient  |
| 15.1 – 20      | 60                        | 40                        | re-equilibration |

Typical chromatogram for solution (3) from the Assay test for Bumetanide Injection as published in BP 2027.



Peak ID: 1: Impurity B. 2: Impurity A. 3: Bumetanide. 4: Impurity C.

|                  |                                                           |
|------------------|-----------------------------------------------------------|
| Column           | XTerra C18 (150 mm x 4.6 mm, 3.5 µm)                      |
| Method Ref.      | Assay for the Bumetanide Injection monograph from BP 2027 |
| Mobile Phase A   | 0.5% v/v formic acid                                      |
| Mobile Phase B   | Methanol                                                  |
| Diluent          | 40% methanol                                              |
| Flow rate        | 1.0 mL/min                                                |
| Column Temp      | 30°C                                                      |
| Injection Volume | 50 µL                                                     |
| Detection        | 254 nm                                                    |

| Gradient       |                           |                           |                  |
|----------------|---------------------------|---------------------------|------------------|
| Time (minutes) | Mobile phase A<br>(% v/v) | Mobile phase B<br>(% v/v) | Comment          |
| 0 – 2          | 60                        | 40                        | isocratic        |
| 2 – 10         | 60 → 20                   | 40 → 80                   | linear gradient  |
| 10 – 15        | 20                        | 80                        | isocratic        |
| 15 – 15.1      | 20 → 60                   | 80 → 40                   | linear gradient  |
| 15.1 – 20      | 60                        | 40                        | re-equilibration |