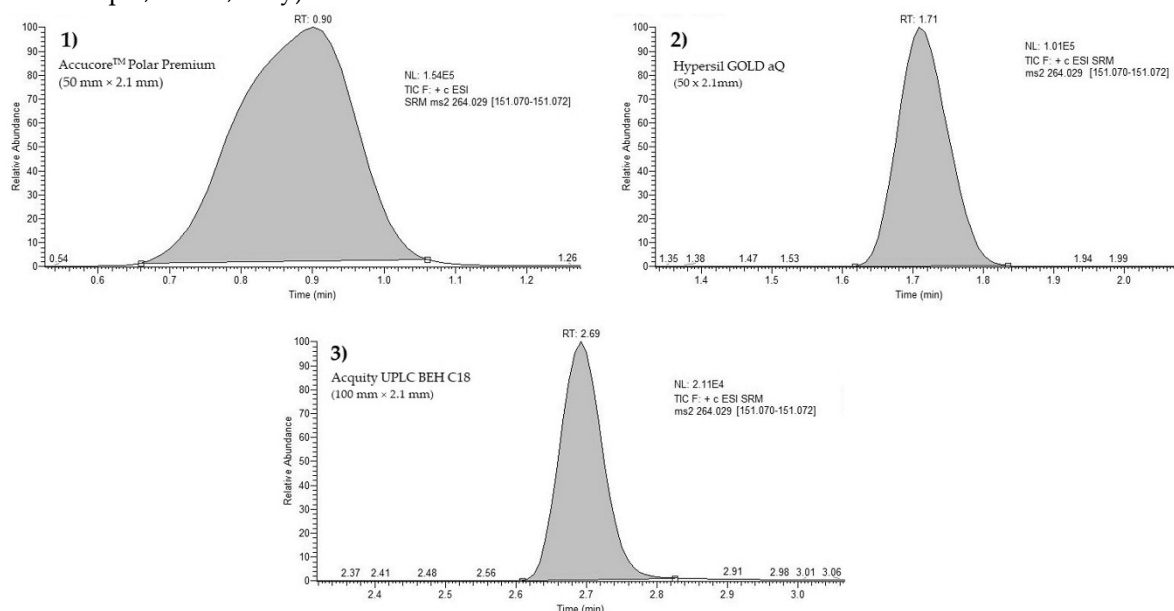


SUPPLEMENTARY MATERIALS

Supplementary Figure S1. Chromatographic peaks obtained in spiked sample of Busulfan 2000 ng/mL with 3 different tested chromatographic columns: 1) Thermo Scientific™ Accucore™ Polar Premium column (50 mm × 2.1 mm, i.d. 2.6 µm, Thermo Fisher Scientific, Milan, Italy), 2) Hypersil GOLD aQ (50 x2.1 mm, i.d. 1.9 µm, Thermo Fisher Scientific, Milan, Italy) and 3) Acquity UPLC BEH C18 (2.1 mm × 100 mm, i.d. 1.7 µm, Waters SpA, Milan, Italy).



Supplementary Table S1. Resolution, symmetry and peak classification parameters set in the peak suitability software evaluated for chromatographic column selection.

| | | |
|---|------------------------------------|----------|
| Resolution Parameters | Resolution Threshold (%) | 90% |
| Simmetry Parameters | Peak height (%) | 50% |
| | Symmetry threshold (%) | 90% |
| Peak classification Parameters | Peak height (%) | 50% |
| | Min peak width (sec) | 1.80 sec |
| | Max peak width (sec) | 3.60 sec |
| Detect tailing | Peak height (%) | 10% |
| | Failure threshold | 2.0 |
| Detect column overload | Peak height (%) | 50 % |
| | Failure threshold | 1.5 |
| Detect baseline clipping | Number of peak for noise detection | 1.0 |
| Detect minimum Signal-to-noise ratio | Signal-to-noise ratio | 3 |

Supplementary Table S2. Results obtained from each chromatographic column tested during method development. Column 1: Thermo Scientific™ Accucore™ Polar Premium column (50 mm × 2.1 mm, i.d. 2.6 µm, Thermo Fisher Scientific, Milan, Italy). Column 2: Hypersil GOLD aQ (50 x2.1 mm, i.d. 1.9 µm, Thermo Fisher Scientific, Milan, Italy). Column 3: Acquity UPLC BEH C18 (2.1 mm × 100 mm, i.d. 1.7 µm, Waters SpA, Milan, Italy).

| | Column 1 | Column 2 | Column 3 |
|------------------------------|-----------------|-----------------|-----------------|
| Symmetrical | Failed | Failed | Passed |
| Resolution | Failed | Passed | Passed |
| Peak Width | Failed | Failed | Failed |
| Tailing | Failed | Failed | Passed |
| Column Overload | Failed | Passed | Passed |
| Baseline Clipping | Failed | Passed | Passed |
| Signal-to-noise ratio | Failed | Passed | Passed |
| Concave | Failed | Passed | Passed |
| Saturation | Failed | Passed | Passed |