***SUPPORTING INFORMATION***

# Optimisation by Design of Experiment of Benzimidazol-2-One Synthesis under Flow Conditions

**Serena Mostarda [1,2,†], Tugçe Gür Maz [3,†], Alessandro Piccinno [1] Bruno Cerra [1,*] and Erden Banoglu [3,*]**

[1] Department of Pharmaceutical Sciences, University of Perugia, Via del Liceo 1, 06123, Perugia, Italy; serena.mostarda@novartis.com (S.M.); alessandro.piccinno@chimfarm.unipg.it (A.P.)
[2] Current affiliation: Novartis Pharma AG, CH-4002 Basel, Switzerland
[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Etiler, 06560, Ankara, Turkey; ztugcegur@gmail.com (T.G.M.)
* Correspondence: bruno.cerra@chimfarm.unipg.it (B.C.); banoglu@gazi.edu.tr (E.B.); Tel.: +39-075-5855120 (B.C.) Tel.: +90-31-2023236 (E.B.)
† These authors contributed equally to this work.

**Figure S1: $^{1}$H-NMR of compound 1 ($d^{6}$-DMSO, 400 MHz)**

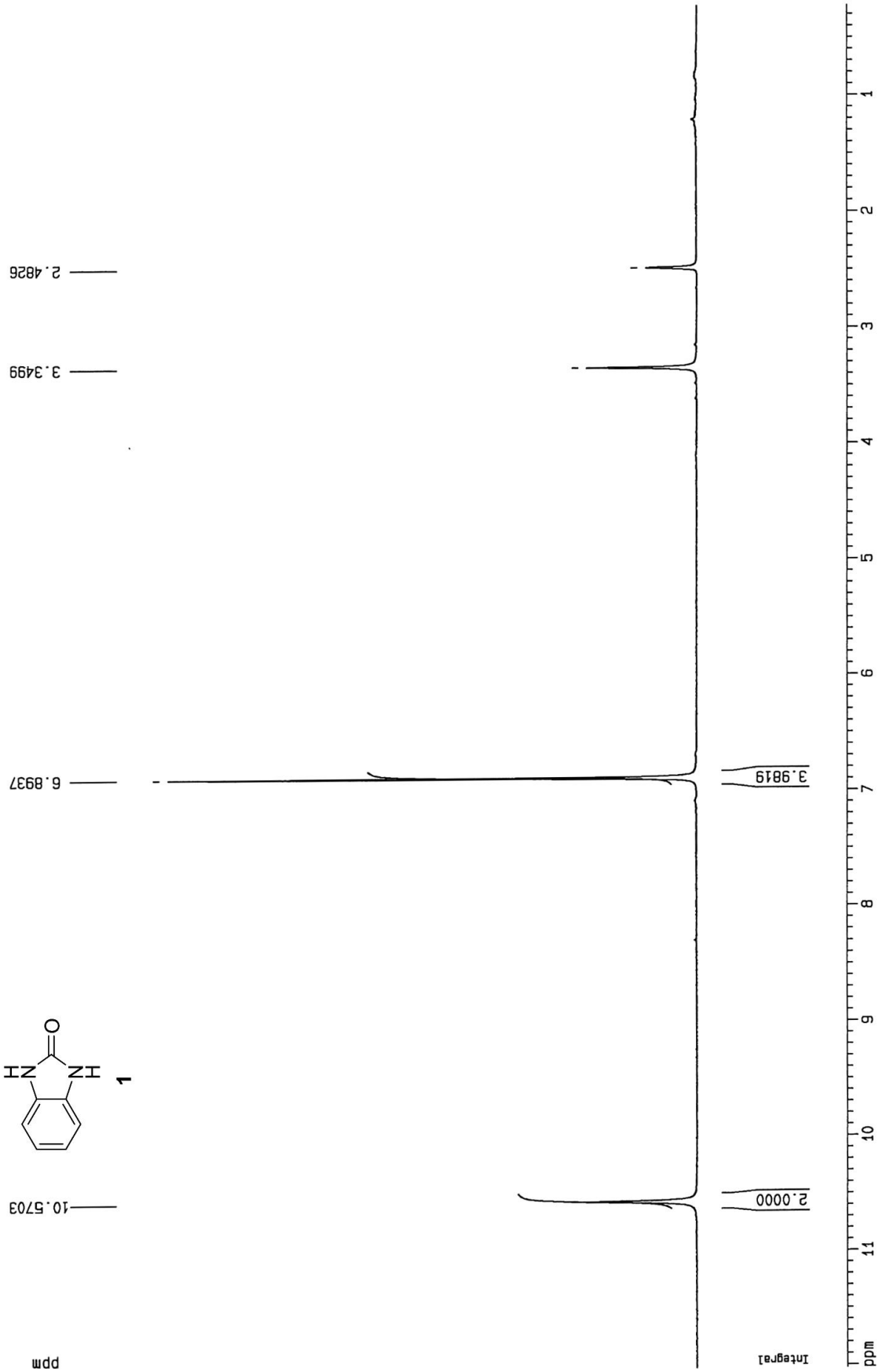


**Figure S2: $^{13}$C-NMR of compound 1 ($d^6$-DMSO, 100.6 MHz)**

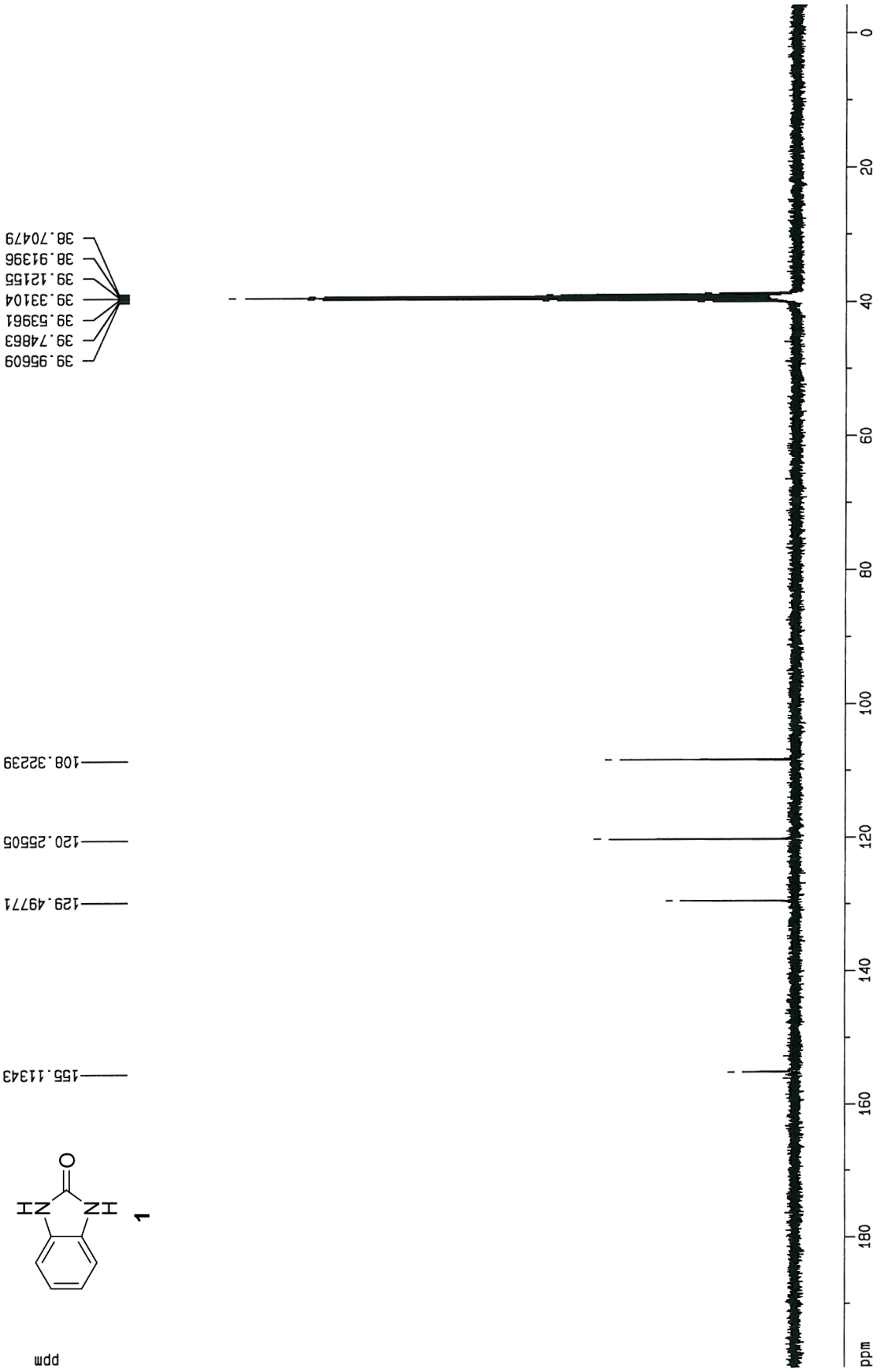


**Figure S3: $^{1}$H-NMR of compound 3 ($CDCl_3$, 400 MHz)**

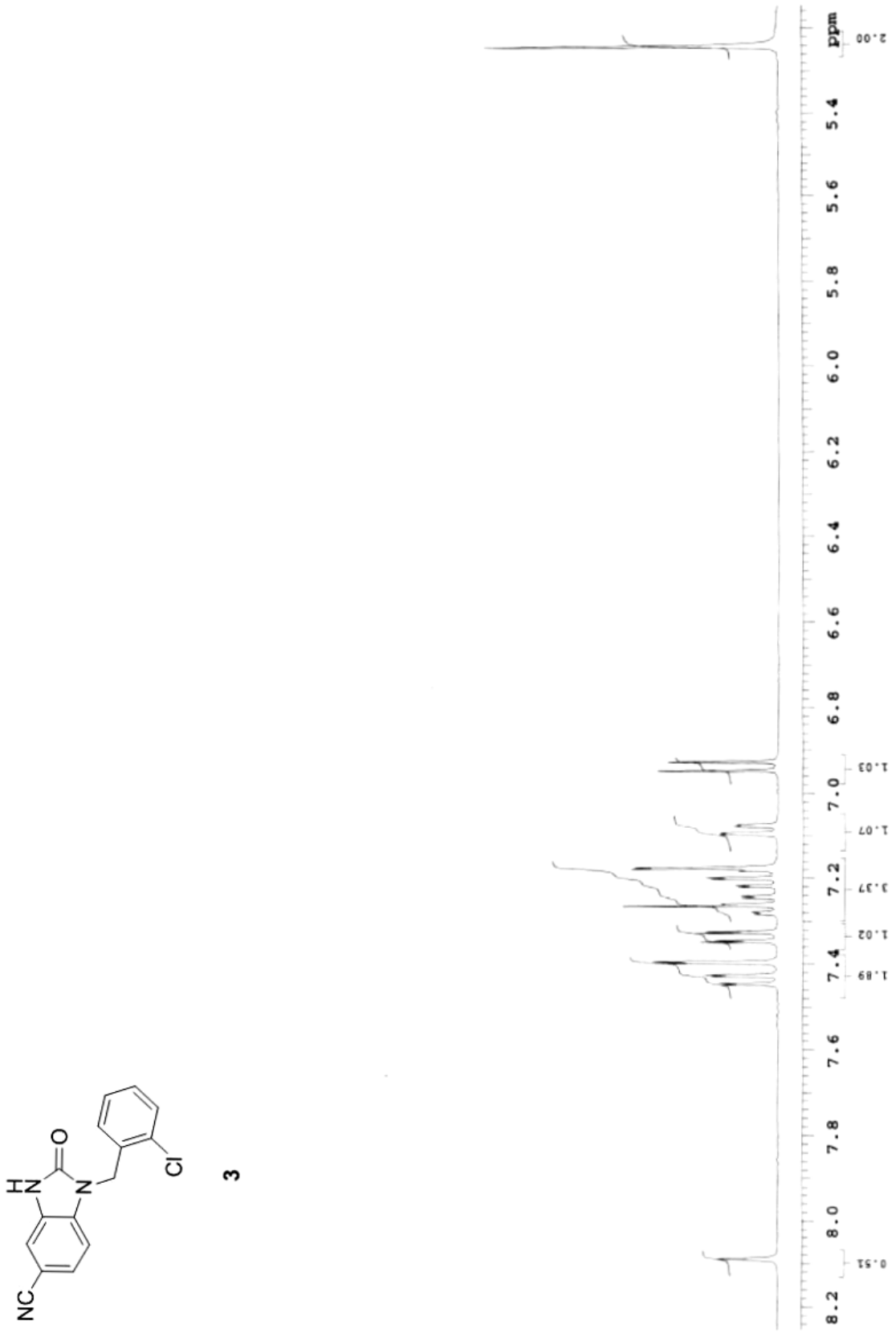


**Figure S4: $^{13}$C-NMR of compound 3 ($d^6$-DMSO, 100.6 MHz)**

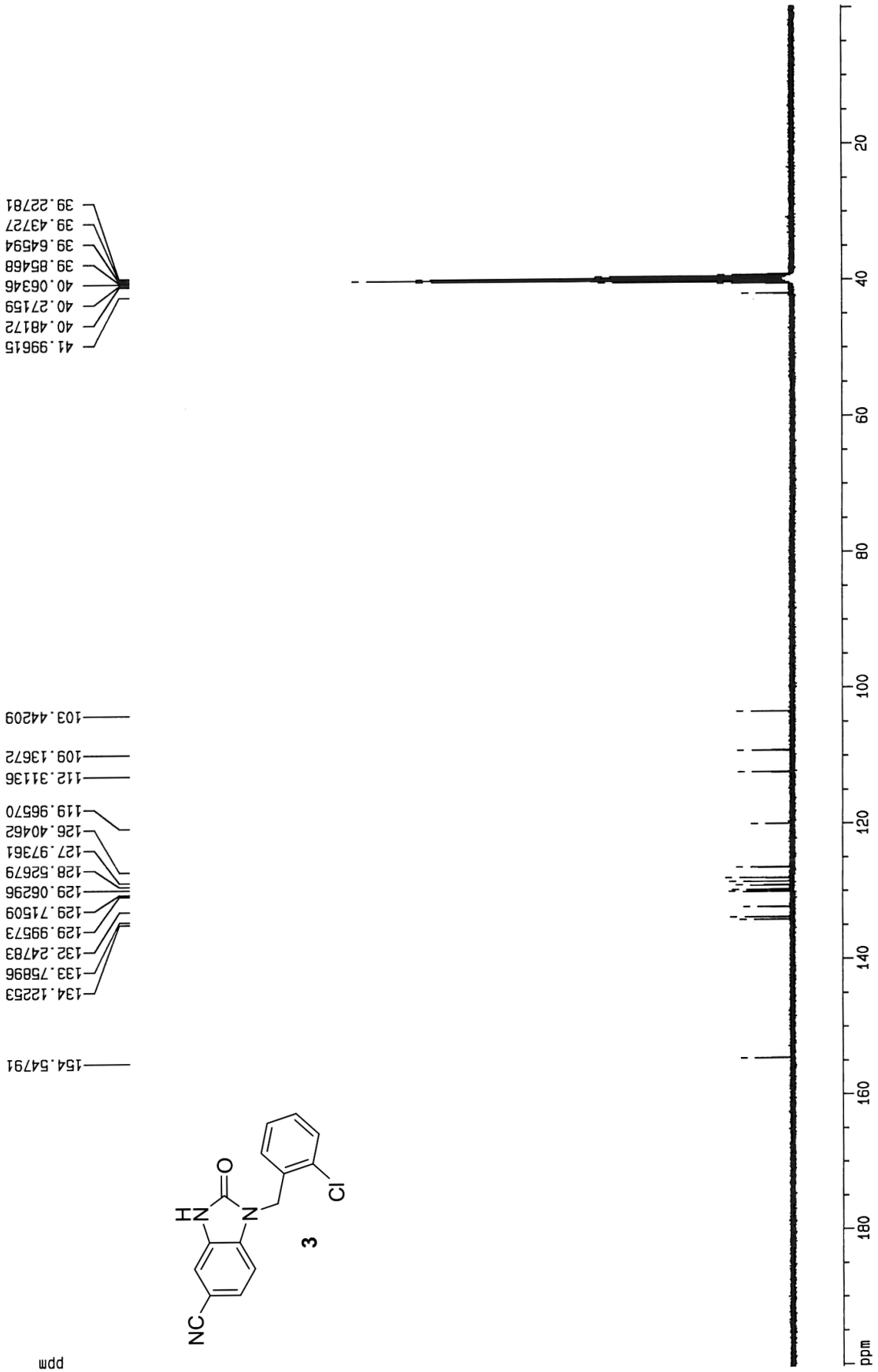
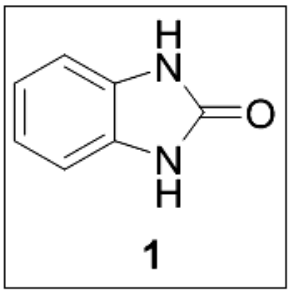


**Figure S5: HPLC calibration curve for compound 1**

| Conc (mM) | Area |
|---|---|
| 0,06 | 43726,808 |
| 0,036 | 30313,024 |
| 0,018 | 16817,372 |
| 0,001 | 821,07 |
| 0,0001 | 134,443 |

| | |
|---|---|
| Slope | 7,41E+05 |
| Intercept | 1,31E+03 |
| $R^2$ | 0,9939 |




1

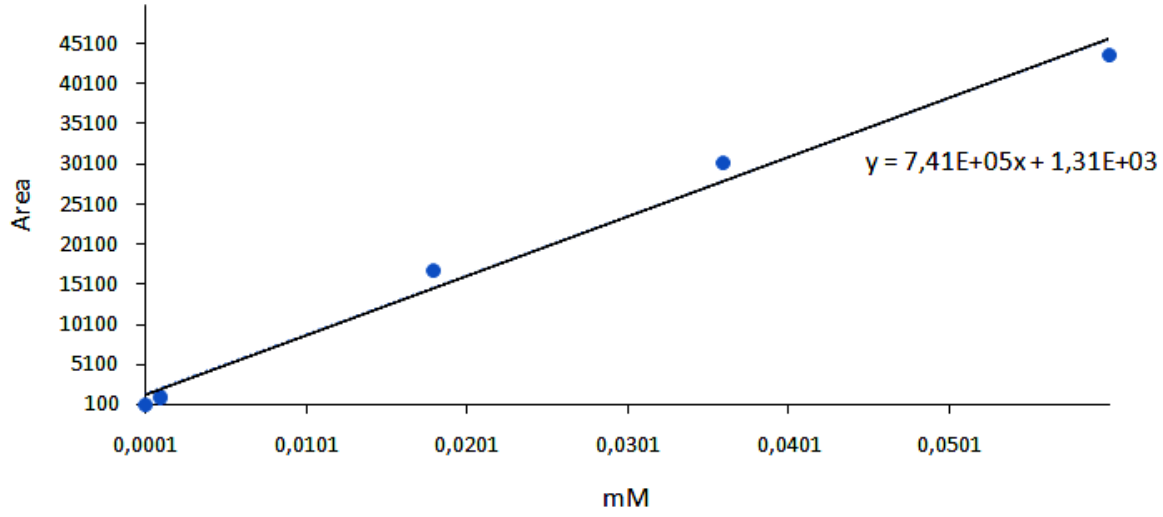
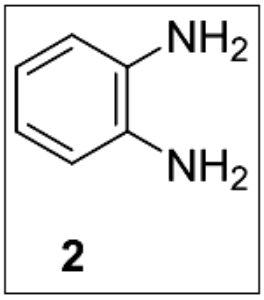


**Figure S6: HPLC calibration curve for compound 2**

| Conc (mM) | Area |
|---|---|
| 0,019 | 29482,197 |
| 0,018 | 28993,424 |
| 0,016 | 25437,047 |
| 0,015 | 23108,462 |
| 0,013 | 21761,275 |

| | |
|---|---|
| Slope | 1,42E+06 |
| Intercept | 2,82E+03 |
| $R^2$ | 0,9817 |




**2**

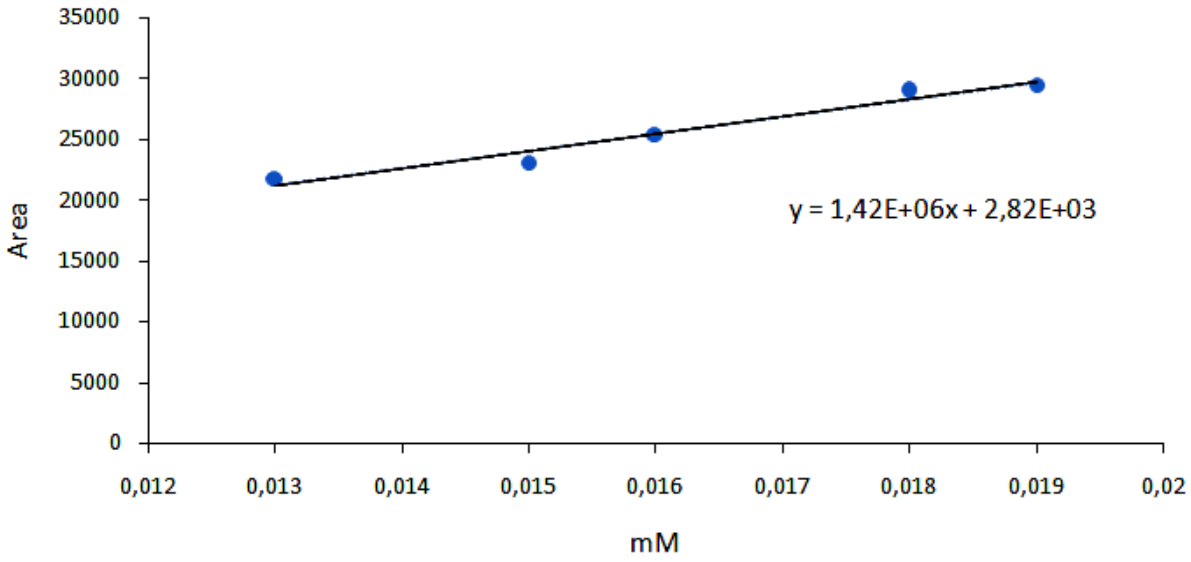
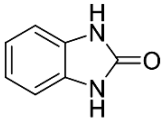


**Figure S7: Overview of the results based on calibration curves**

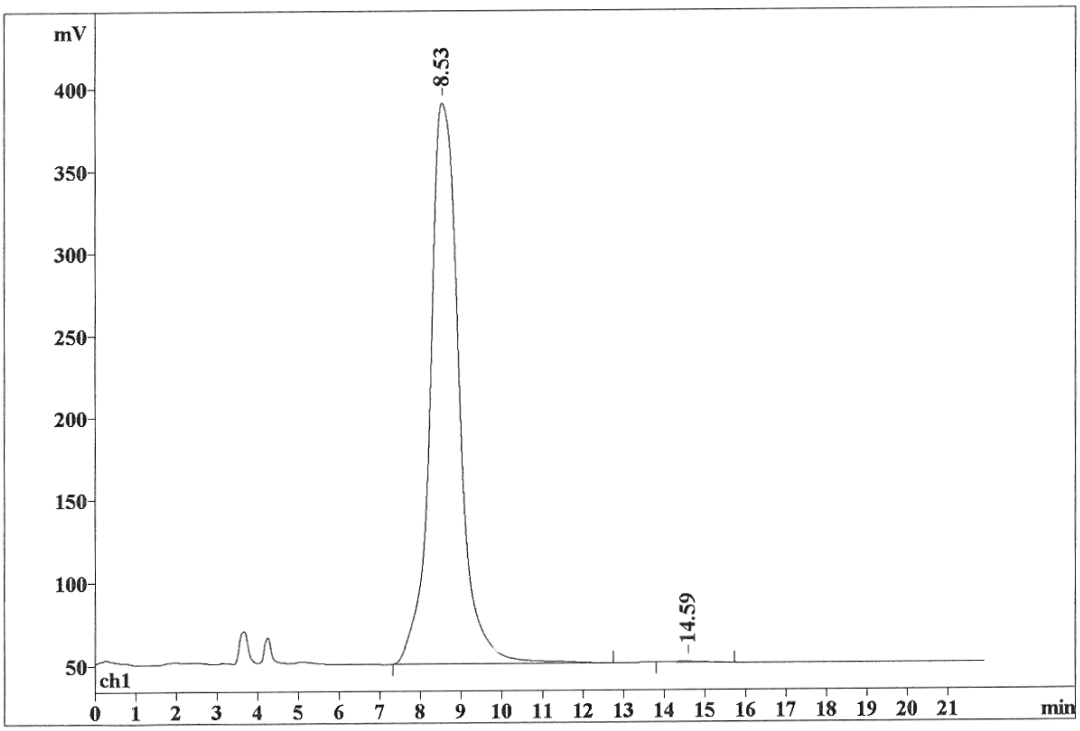
| | Slope | 1415994 | | | Slope | 740799 | |
|---|---|---|---|---|---|---|---|
| | Intercept | 2817 | | | Intercept | 1309 | |
| | $R^2$ | 0,9817 | | | $R^2$ | 0,9939 | |
| | o-Phenylenediamine (2) | | | | Benzimidazolone (1) | | |
| | Area | mM | % | | Area | mM | % |
| Run 1 DoE Table 3 | 29969 | 0,01917 | 89,7 | | 2939 | 0,002200 | 10,3 |
| Run 2 DoE Table 3 | 35533 | 0,02310 | 96,8 | | 1880 | 0,000771 | 3,2 |
| Run 3 DoE Table 3 | 27092 | 0,01714 | 91,7 | | 2461 | 0,001555 | 8,3 |
| Run 4 DoE Table 3 | 27863 | 0,01769 | 67,8 | | 7537 | 0,008407 | 32,2 |
| Run 5 DoE Table 3 | 26964 | 0,01705 | 72,6 | | 6085 | 0,006447 | 27,4 |
| Run 6 DoE Table 3 | 33891 | 0,02194 | 100,0 | | 57 | 0,000000 | 0,0 |
| Run 7 DoE Table 3 | 19388 | 0,01170 | 73,0 | | 4513 | 0,004325 | 27,0 |
| Run 8 DoE Table 3 | 36555 | 0,02383 | 100,0 | | 183 | 0,000000 | 0,0 |
| Run 9 DoE Table 3 | 41151 | 0,02707 | 39,3 | | 32272 | 0,041796 | 60,7 |
| Run 10 DoE Table 3 | 25194 | 0,01580 | 87,4 | | 2993 | 0,002273 | 12,6 |
| Run 11 DoE Table 3 | 8126 | 0,00375 | 22,3 | | 10974 | 0,013046 | 77,7 |
| Run 12 DoE Table 3 | 33615 | 0,02175 | 93,6 | | 2403 | 0,001476 | 6,4 |
| Run 13 DoE Table 3 | 37313 | 0,02436 | 100,0 | | 11 | 0,000000 | 0,0 |
| Run 14 DoE Table 3 | 6551 | 0,00264 | 16,2 | | 11447 | 0,013685 | 83,8 |
| Run 15 DoE Table 3 | 19021 | 0,01144 | 67,7 | | 5345 | 0,005448 | 32,3 |
| Run 16 DoE Table 3 | 8918 | 0,00431 | 13,4 | | 21881 | 0,027769 | 86,6 |
| Run 17 DoE Table 3 | 18506 | 0,01108 | 61,9 | | 6369 | 0,006829 | 38,1 |
| Run 18 DoE Table 3 | 18330 | 0,01096 | 61,7 | | 6340 | 0,006790 | 38,3 |
| Run 19 DoE Table 3 | 19108 | 0,01150 | 74,6 | | 4217 | 0,003926 | 25,4 |
| Check point Table 5 | 3402 | 0,00041 | 2,0 | | 16284 | 0,020214 | 98,0 |

**Figure S8: HPLC chromatogram of compound 1 (reference standard)**

| | |
|---|---|
| SAMPLE: | Benzimidazol-2-one0.018M |
| : | |
| Vial number: | 4 |
| Volume: | 20.0 µl |
| Dilution: | 1.00 |
| Amount: | 1.0000 |
| COLUMN: | Ultra II Aqueous |
| Size: | 4.6 x 250 mm |
| Number: | |
| Part.size: | 5.0 µm |
| ELUENT: | MeOH/H2O-25/75 (v,v) + DEA 0.1% |
| Flow: | 0.00 mL/min |
| Temperature: | -273.0°C |
| Pressure: | -145.0 psi |



1

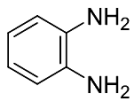



Quantitation method: Custom

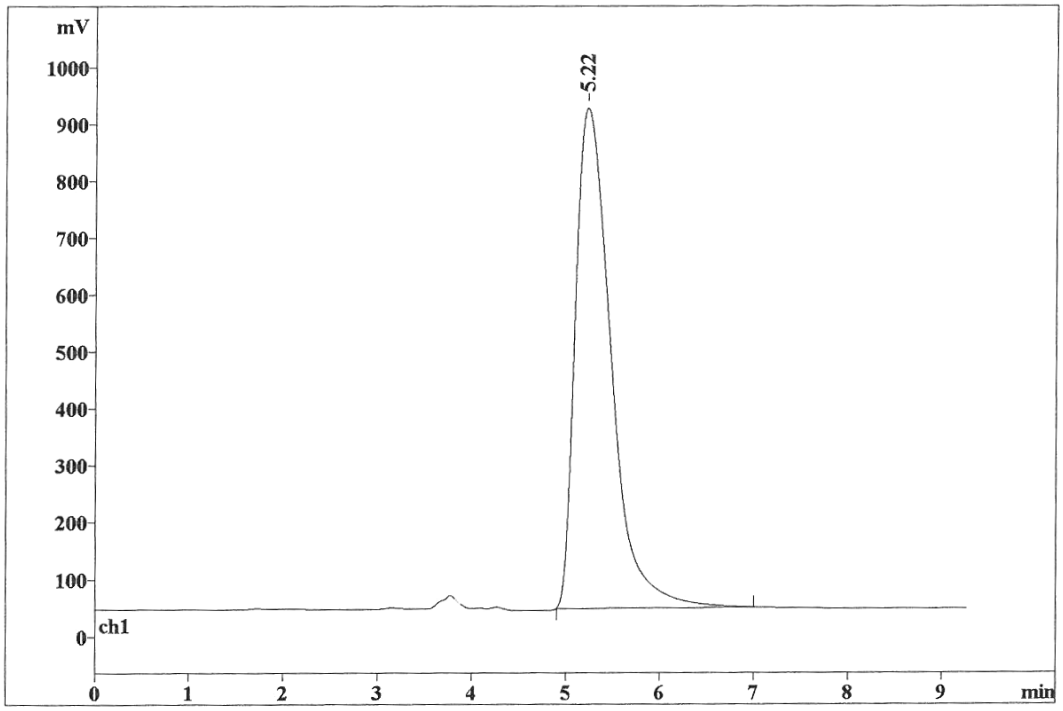
| Retention min | Width/2 min | Height mV | Height % | Area mV*sec | Area % |
|---|---|---|---|---|---|
| 8.53 | 0.708 | 340.48 | 99.84 | 16817.372 | 99.83 |

**Figure S9: HPLC chromatogram of compound 2 (reference standard)**

| | |
|---|---|
| SAMPLE: | Diamine0.015M |
| : | |
| Vial number: | 4 |
| Volume: | 20.0 µl |
| Dilution: | 1.00 |
| Amount: | 1.0000 |
| COLUMN: | Ultra II Aqueous |
| Size: | 4.6 x 250 mm |
| Number: | |
| Part.size: | 5.0 µm |
| ELUENT: | MeOH/H2O-25/75 (v,v) + DEA 0.1% |
| Flow: | 0.00 mL/min |
| Temperature: | -273.0°C |
| Pressure: | -145.0 psi |




**2**

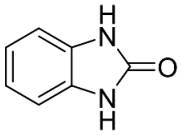



Quantitation method: Custom

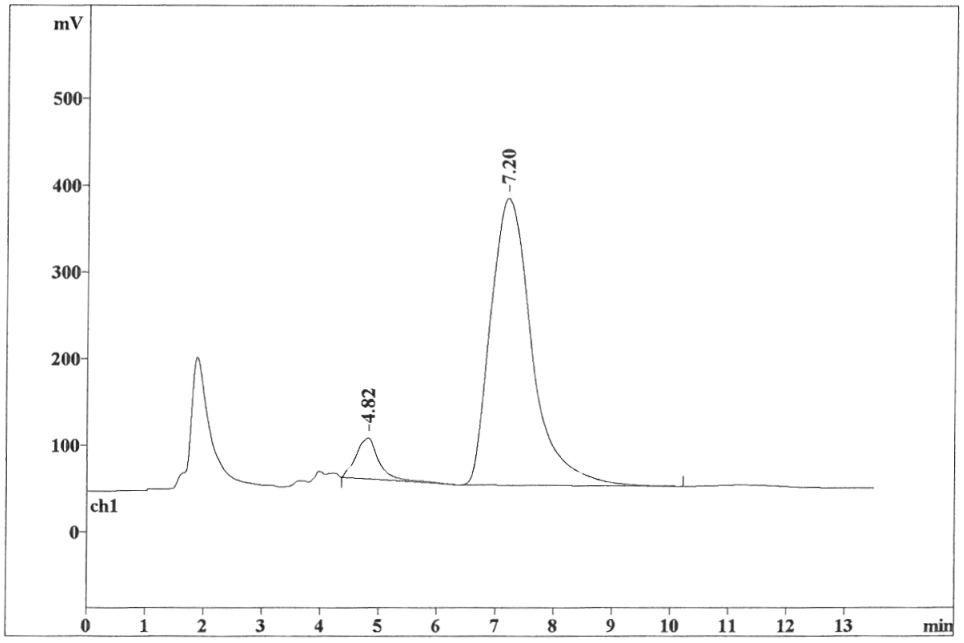
| Retention min | Width/2 min | Height mV | Height % | Area mV*sec | Area % |
|---|---|---|---|---|---|
| 5.22 | 0.399 | 878.83 | 100.00 | 23108.462 | 100.00 |

**Figure S10: HPLC chromatogram of crude 1 obtained by optimised flow conditions (Table 5)**

| | |
|---|---|
| Vial number: | 4 |
| Volume: | 20.0 µl |
| Dilution: | 1.00 |
| Amount: | 1.0000 |
| COLUMN: | Ultra II Aqueous |
| Size: | 4.6 x 250 mm |
| Number: | |
| Part.size: | 5.0 µm |
| ELUENT: | MeOH/H2O-25/75 (v,v) + DEA 0.1% |
| Flow: | 0.00 mL/min |
| Temperature: | -273.0°C |
| Pressure: | -145.0 psi |




**1**

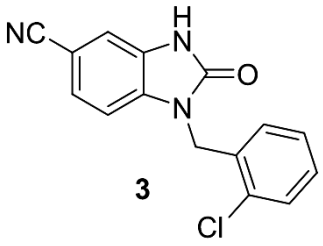



Quantitation method: Custom

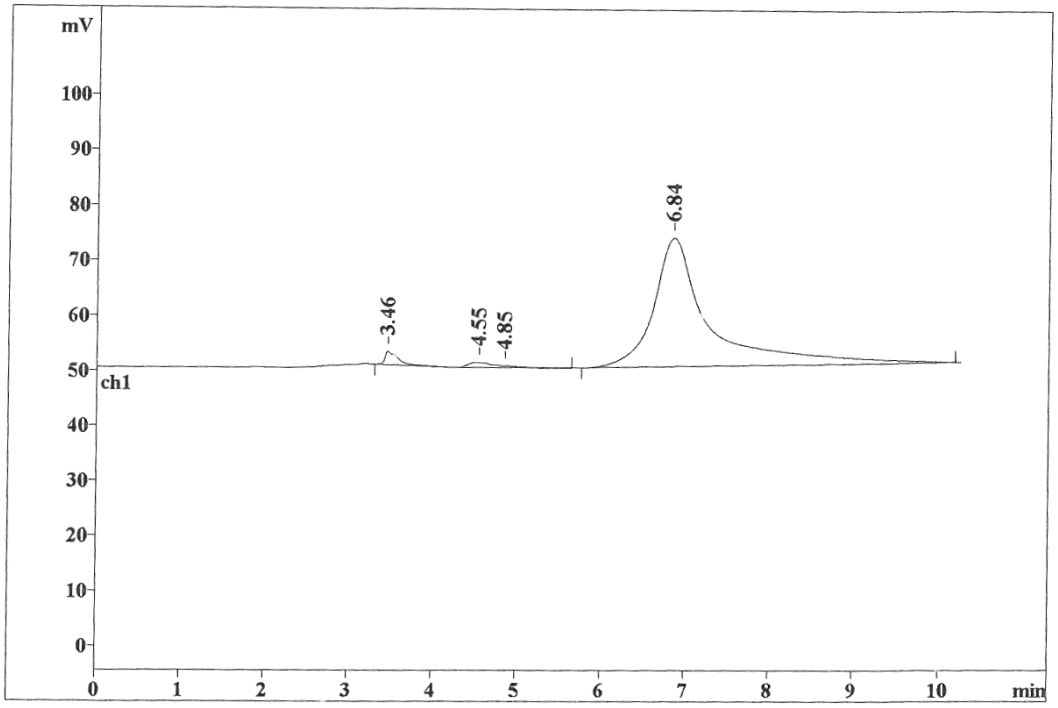
| Retention min | Width/2 min | Height mV | Height % | Area mV*sec | Area % | k |
|---|---|---|---|---|---|---|
| 4.82 | 0.387 | 47.22 | 12.48 | 1312.609 | 7.46 | 0.00 |
| 7.20 | 0.734 | 331.05 | 87.52 | 16284.211 | 92.54 | 0.00 |
| 13.51 | 0.560 | 378.27 | 100.00 | 17596.821 | 100.00 | 0.00 |

**Figure S11: HPLC chromatogram of crude 3 obtained by optimised flow conditions (Figure 4)**

| | |
|---|---|
| Volume: | 20.0 µl |
| Dilution: | 1.00 |
| Amount: | 1.0000 |
| COLUMN: | Ultra II Aqueous |
| Size: | 4.6 x 250 mm |
| Number: | |
| Part.size: | 5.0 µm |
| ELUENT: | MeOH/H2O-25/75 (v,v) + DEA 0.1% |
| Flow: | 0.00 mL/min |
| Temperature: | -273.0°C |
| Pressure: | -145.0 psi |




3




Quantitation method: Custom

| Retention min | Width/2 min | Height mV | Height % | Area mV*sec | Area % | k |
|---|---|---|---|---|---|---|
| 3.46 | 0.158 | 2.48 | 9.09 | 28.257 | 2.45 | 0.00 |
| 4.53 | 0.352 | 0.94 | 3.45 | 19.044 | 1.65 | 0.00 |
| 4.85 | 0.148 | 0.41 | 1.49 | 5.298 | 0.46 | 0.00 |
| 6.84 | 0.492 | 23.45 | 85.98 | 1100.167 | 95.44 | 0.00 |