D1:

Online dual gradient reversed-phase / porous graphitized carbon nanoHPLC for proteomic applications

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File contains the gradient program used for the U3000 dual gradient separations. The command style is from Chromeleon Software (Dionex, Germany).

```
        UV_VIS_1.Wavelength =     214 [nm]
        UV_VIS_1.Step =     0.20 [s]
        UV_VIS_1.Average =          On
        UV_VIS_2.Wavelength =     295 [nm]
        UV_VIS_2.Step =     0.20 [s]
        UV_VIS_2.Average =          On
        ColumnCompartment1.ValveLeft =  10_1
        ColumnCompartment1.ValveRight =          1_2      ; column 1 is in line with UV
        ColumnCompartment2.ValveLeft =  10_1

 0.000  Autozero
        NanoPump1.Flow =   0.220 [µl/min]
        NanoPump1.%B =     7.0 [%]
        NanoPump1.%C =     0.0 [%]
        NanoPump2.Flow =   0.260 [µl/min]
        NanoPump2.%B =     7.0 [%]
        NanoPump2.%C =     0.0 [%]

        Inject

        LoadingPump1.Flow =         7 [µl/min]
        LoadingPump1.%B =           0.0 [%]
        LoadingPump1.%C =           0.0 [%]
        NanoPump1.Flow =   0.220 [µl/min]
        NanoPump1.%B =     7.0 [%]
        NanoPump1.%C =     0.0 [%]
        NanoPump2.Flow =   0.260 [µl/min]
        NanoPump2.%B =     7.0 [%]
        NanoPump2.%C =     0.0 [%]


 0.100  LTQ.State       On

 1.000  LTQ.State       Off


 7.000  ColumnCompartment1.ValveLeft =  1_2
        NanoPump1.%B =     7.0 [%]
        LoadingPump1.Flow =         7 [µl/min]
        NanoPump2.%B =     7.0 [%]

 8.000  NanoPump1.%B =     7.0 [%]
        NanoPump2.%B =     7.0 [%]
        LoadingPump1.Flow =         1 [µl/min]


38.000  NanoPump1.%B =     50.0 [%]


42.000  NanoPump1.%B =     95.0 [%]
44.000  NanoPump2.%B =     7.0 [%]
```

45.000 NanoPump2.%B = 7.0 [%]
46.000 LoadingPump1.Flow = 1 [µl/min]
48.000 LoadingPump1.Flow = 7 [µl/min]

50.000 NanoPump1.%B = 95.0 [%]
ColumnCompartment2.ValveLeft = 1_2
ColumnCompartment1.ValveRight = 6_1

55.000 NanoPump1.%B = 50.0 [%]
NanoPump2.%B = 7.0 [%]

80.000 NanoPump1.%B = 7.0 [%]
ColumnCompartment1.ValveLeft = 10_1

85.000 NanoPump2.%B = 40.0 [%]

90.000 NanoPump2.%B = 90.0 [%]

95.000 NanoPump2.%B = 90.0 [%]

100.000 NanoPump2.%B = 7.0 [%]
NanoPump1.%B = 7.0 [%]
ColumnCompartment2.ValveLeft = 10_1

110.000 NanoPump1.%B = 7.0 [%]
NanoPump2.%B = 7.0 [%]
LoadingPump1.Flow = 7 [µl/min]

120.000
NanoPump1.Flow = 0.220 [µl/min]
NanoPump1.%B = 7.0 [%]
NanoPump1.%C = 0.0 [%]
NanoPump2.Flow = 0.260 [µl/min]
NanoPump2.%B = 7.0 [%]
NanoPump2.%C = 0.0 [%]
ColumnCompartment1.ValveRight = 1_2
ReleaseExclusiveAccess
End