

Quality Assurance Dose Response Experiments - Identity and Purity Analysis using HPLC-MS

Abbreviations:

CBCS = Chemistry Biology Consortium Sweden

ES⁺ = Electro spray, positive mode

MilliQ = water treated with ultraviolet light and run through a particle filter

HPLC = High pressure liquid chromatography

TFA = Trifluoroacetic acid

Description:

Dose Response experiment which describes the change in effect of an organism (e.g. cells) caused by differing levels of exposure to a compound after a certain exposure time, is a study to determine the level of activity of selected compounds confirmed as hits in earlier screens.

The purpose of the procedure is to assure the quality of the data generated from the Dose Response experiment by confirming the identity and purity of the selected compounds. Purity > 85% is required to pass the quality control, if purity is less or mass peak is not found the compound will be assigned as a suspect until further analysis can be done e.g alternative LC-MS method or NMR.

Procedures:

Scilifelab Compound Center will perform identity- and purity analysis on HPLC UV/MS and validate the data whenever a Dose Response experiment is fulfilled.

From the selected compounds to be part of the Dose Response experiment, 5 µl of 10mM DMSO solution is transferred to a glass vial or on a 96 well polypropylene plate or 500 nL is transferred to a 96 well polypropylene plate. The 5 µL samples are diluted with 50 µl of methanol or acetonitrile, the 500 nL samples are diluted with 10 µL methanol or acetonitrile and then placed into the HPLC-MS for analysis.

Summary of Method:

Methods and specification of instrument and validation is fully described in the electronic notebook (ELN KI) EXP-22-DT9001 and (ELN UU) SOP_UU_BK0902.

Equipment needed:

Agilent 1260 Infinity II HPLC UV/MS

Systems: "Caesar" or "Cleopatra"

Methods:

ES⁺ in 0.1% TFA in MilliQ water as mobile phase A and methods QA1097-3 or A1097-3.

Compounds analyzed in method above not giving satisfactory data are re-analyzed using method:

ES⁺ in 10mM NH₄HCO₃ pH 10 in MilliQ water as mobile phase A and method QX1097-3.

Validating data:

The data is provided in a report with an automated integration of the UV-response and purity. The result is controlled and if any deviation from expected outcome a meticulous investigation of the UV-response and mass spectrometry is made manually.

Actions and future aim:

All results will be saved in our database platform, Scarab. All raw data will be saved at \\storage3.ad.scilifelab.se\NMR400\data\Caesar\Asa IP.

Compounds that meet the quality requirements will not undergo any further actions. Compounds not meeting the quality requirements will be reported to the scientist responsible for the experiment. The aim is to assure the quality of the Dose Response experiments to guarantee the generated result as reliable data.