

<i>OREGIN Standard Operating Procedure</i>	
Purpose	To determine oil content in Brassica seeds using NMR
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NMR analysis for oil content in *Brassica* seeds

- The Method sheet for MQC-23-27 Oil and moisture content in seeds (ISO 10565) (enquire @ www.Oxford-Instruments.com) is followed.
- See also the Application note at:

http://www.oxford-instruments.com/wps/wcm/resources/file/eb039d40f513410/Oil_moisture_seeds.pdf

Equipment used (at JIC): Oxford Instruments NMR benchtop analyser: MQC-23-25 magnet with 10 mm probe.

MultiQuant software v5.1 (RI Calibration, RI Analysis and EasyCal applications)

NOTE: Moisture content of calibration and measurement seed samples must be <10%.

Summary method:

1. The EasyCal application for 'oil and moisture in seeds' is used to generate a new calibration: A tuning sample is used to optimise NMR parameters and then a calibration is generated, checked and accepted using the following Canola seed standards:

Standard ID	Ref. Value	Mass (mg)
cl1	39.76	209
cl2	42.2	210.4
cl3	43.67	213
cl4	46.12	204
cl5	48.18	205
cl6	49.35	209.2
cl8	52.08	206.2

2. For sample analyses, open 'RI analysis' in the MultiQuant folder and select the correct calibration file.
3. Accurately weigh each ~200 mg seed sample (the glass tube is supported in a 100 ml flask on a top loading balance). The same glass tube is used for all samples.
4. Enter the sample mass and sample ID on the screen. Click 'ok' and follow the instructions to insert the sample.
5. A replicated randomised block design is used to analyse the seed samples, incorporating the inclusion of the Canola seed standards and three additional

reference standards of *B. napus*: TapidorDH (GK060284, 202.8 mg), Ningyou 7 (GK060276, 205.9 mg) and R-O-18 (GK060911a, 205.7 mg). This allows for machine/day/time variation to be accounted for. Auto-tuning (with the tuning sample) is also requested intermittently.

6. When scan counting is completed, NMR results are displayed and a request to remove the sample is shown on the screen.
7. Data is saved and downloaded as .txt files.

Additional information:

- Initially, tests to address machine, person and sample tube variability, as well as, independent vs. re-sampled replicates and repeatability, were undertaken.
- The same sample tube was used for each 'set' of seed.
- High person variability caused by the time taken by an individual to place the sample into the NMR is overcome by placing the tube into the NMR prior to starting the measurement.
- To determine oil content in 205 BnaDFFS seed accessions (as above) took ~32 man hours.