HUMAN NUTRIENT METHODS

Single-Laboratory Validation Study of a Rapid TD-NMR Method for Quantitation of Total Fat in Sunflower Oil Powder

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Abstract

Background: A rapid total fat quantitation method for sunflower oil powder was developed using time-domain nuclear magnetic resonance (TD-NMR). Currently, industry has three major methods for the total fat quantitation: gravimetric analysis after ether extraction (AOAC Methods **933.05** and **989.05**), gas chromatography with flame ionization detector (GC-FID; AOAC Method **996.06**), and High-resolution NMR. The gravimetric analysis method takes a day using highly flammable solvents, and the GC-FID method takes two days requiring harsh chemicals for hydrolyzation, extraction, and methylation. The High-resolution NMR spectroscopy method requires simpler sample preparation and shorter analysis time compared to the other two methods. Often, the only required sample preparation step is to dissolve a sample in a solvent. The acquisition time depends on types of analyzing nuclei and sample. The vegetable oil analysis by ^{13}C NMR takes about 4 h per sample. ^{1}H NMR usually takes less time to analyze. In contrast, the TD-NMR relaxometry method takes only 1 h to prepare and analyze samples if the test is for total fat only. The acquisition time is 40 s per sample, and samples are analyzed "as is". A rapid analysis method in a quality control laboratory is very crucial for laboratory efficiency in releasing products. In this paper, a single-laboratory validation study is described for a rapid TD-NMR method to quantitate total fat in sunflower oil powder.
Objective: This validation work is to provide documented evidence for the method validity as well as the method performance.
Method: The method used a Bruker *minispec mq-20 NMR analyzer*® with *minispec plus*® software. A Hahn echo pulse program was used in the method to collect spin echo signal to determine total fat content.
Results: The *linearity/range* result from 10 standards (0, 21, 42, 63, 83, 92, 100, 108, 117, and 125%) has coefficients of determination (R^2) of 1.0000. The 100% level is 1.2 g-fat in 2.5 g sample, which is targeted fat content in a sunflower oil powder raw material. The method is specific for the quantitation of total fat in sunflower oil powder with no background interference from the matrix. The precision result of the 6 replicate samples at 100% level is 0.3% RSD. The accuracies measured from triplicate analysis of 80, 100, and 120% sample matrices are 100, 100, and 100% average recoveries, respectively. The ruggedness of the test method is 0.4% RSD of 12 analysis from 2 analysts (6 results from each analyst) on the different days.
Conclusions: The test method is proven to be specific, linear, precise, accurate, rugged, and suitable for the intended use of quantitative analysis for total fat in sunflower oil powder.
Highlights: Traditional methods of gravimetric or GC-FID for total fat analysis of raw materials require lengthy sample preparation and experiment time. Laboratory needs to spend a day to perform gravimetric analysis following ether extraction method and 2 days for the GC-FID method. In addition, these test methods use highly flammable and harsh

chemicals that generate hazardous chemical wastes. These hazardous wastes are harmful to analysts and environments. In contrast, the TD-NMR method is safe, environmentally friendly, and fast. Therefore, TD-NMR is a preferred method for quality control laboratories.

Time-domain nuclear magnetic resonance (TD-NMR) has been used in milk quality assessment (1), beef differentiation for tracing the sex and bull race (2), quantification of fat and water content in cheese (3), morphology of polymer blends determination (4), and quantitation of gasoline adulteration (5). We have developed and validated a TD-NMR method for rapid analysis of total fat for sunflower oil powder raw material that has enhanced quality control testing efficiency .

Traditionally, there are three methods in the industry that can be used for total fat quantitation for sunflower powder raw material, which are the gravimetric method with ether extraction for total fat (AOAC Methods 933.05 and 989.05), the gas chromatography with flame ionization detector (GC-FID) method (AOAC Method 996.06), and the NMR spectroscopy method. However, these methods are not derived from the total fat test of high oleic sunflower oil powder. AOAC Method 933.05 is for fat in cheese, AOAC Method 989.05 is for fat in milk, AOAC Method 996.06 is a general method for individual fatty acids in food, and the NMR spectroscopy method is used to determine unsaturated fatty acids rapidly and accurately. It is desirable to develop a method to rapidly quantitate total fat in sunflower oil powder in a quality control lab for raw material release to support production. This led us to the development and validation of a TD-NMR method.

There are great advantages using the TD-NMR spectroscopy technique over the gravimetric method after ether extraction or the GC-FID method. The TD-NMR method is not only fast for sample turnaround time as the sample is analyzed "as is" without further preparation steps, but often the results obtained are more accurate. The gravimetric method extracts fat in a sample using ethyl ether and petroleum ether. In the process of extraction, any ether-soluble component other than fat can be dissolved in ether and falsely increase fat content value. For the GC-FID method, multiple sample preparation steps are required including hydrolyzation, extraction, and methylation. Fat can be lost during multiple sample preparation and transferring steps leading to falsely decreased fat content value. The TD-NMR method has very simple sample preparation, and analysis is rapid without chemical solvent wastes.

The NMR spectrometer is a great instrument to determine fat rapidly and accurately, and the early application of proton NMR in determining fatty acid compound was published in 1959 (6). NMR uses different nuclei (^{1}H , ^{13}C, and ^{31}P) spin state to determine unsaturated lipids in animals and different plant parts (seeds, fruits, and nuts) (7). It determines ω-3 polyunsaturated fatty acids content in raw, cooked, and canned fish (8), and the accuracy is comparable to GC (9–11).

Although high-resolution NMR has great ability in determining fatty acid contents accurately, the instrument we have in our lab, TD-NMR, has its own unique advantages. TD-NMR is significantly cheaper and smaller than the high-field NMR. It does not require much space, and its magnetic stray field is smaller than the high-resolution NMR. It does not require cryogens, so the maintenance cost is lower. The operation of instrument and data interpretation is simple, so sophisticated training is not necessary. One can easily obtain highly accurate total fat result with a TD-NMR instrument.

In this study, we have developed a TD-NMR method for the determination of total fat in sunflower oil powder. This test method has gone through a protocol-driven method validation that demonstrates linearity/range, specificity, precision, accuracy, and ruggedness and fits for purpose of its application for rapid quantitation of total fat in sunflower oil powder raw materials.

Experimental

Principle

This method uses the "Hahn Echo" pulse program with two radio frequency (RF) pulses. The pulse program is composed of application of a 90° excitation pulse, waiting time, application of 180° refocusing pulse, and collection of an echo signal after the same amount of waiting time. The diagram of the Hahn Echo pulse program is shown in Figure 1. The 90° excitation pulse will put sample spins in the transverse plane, and a short amount of waiting time will fully relax the solid phase, which has a shorter T_1 time than the liquid phase. Then, the 180° pulse will refocus the spin magnetization in the transverse plane and create an echo signal (12, 13). The echo signal is measured as a percent value. The receiver gain value has been set for the highest concentrated standard to have about an 80% signal value.

The echo signal solely represents the fat content of the sample since the NMR signal decay of bound water is within a few hundreds of microseconds (14). Any moisture content in sunflower oil and matrix will decay before taking an echo signal of the fat, so it will not affect the test result accuracy. The sunflower oil powder sample tested in this study contains ≤4% moisture from a separate test method.

This TD-NMR method is validated for linearity/range, specificity, precision, accuracy, and ruggedness. The details of the test procedure and the method validation are described below .

Reagents and Materials

(a) Sunflower seed oil, analytical standard, Sigma Aldrich, Cat. No. 47123.
(b) Sunflower seed oil from *Helianthus annuus,* Sigma Aldrich, Cat. No. S5007.
(c) Daily check sample, rapeseed, Cat. No. E1405213, Bruker.
(d) Maltodextrin, ADM Specialty (as sunflower oil raw material matrix).
(e) Starch, pregelatinized corn, Sweetener Products Co.
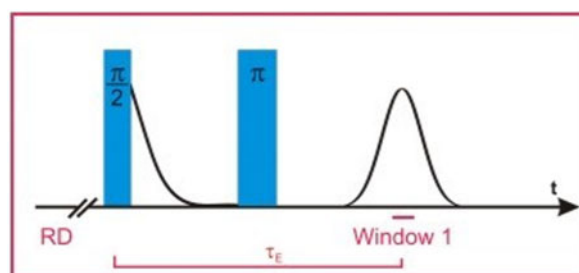

Figure 1. Hahn Echo pulse program.

(f) Kimwipes® tissue paper, Kimberly-Clark Professional.
(g) Glass sample tube with caps, 180 mm × 17.75 mm, Bruker, Cat. No. 1824512.
(h) Glass inserts, 45 mm × 15 mm, Bruker, Cat. No. 65406.
(i) Plug for TD-NMR glass tube, Bruker, Cat. No. E1405258_01.

Equipment

(a) Minispec mq-20 NMR analyzer® spectrometer (20 MHz).
(b) Minispec plus® NF software, Bruker.
(c) Analytical balance.
(d) Heating block.

Procedure

(a) *Matrix preparation.*—Weigh about 45 g of maltodextrin and 19 g of starch and mix them together to make it homogeneous.
(b) *Calibration curve preparation.*—Prepare 10 different standards at concentration of 0, 21, 42, 63, 83, 92, 100, 108, 117, and 125% by the following procedure. Place a sheet of Kimwipes® tissue paper in a glass insert, and add sunflower seed oil analytical standard on the tissue paper. Add only a sheet of tissue paper to a glass insert for 0% standard concentration sample. Add the glass insert into a sample tube and position a plug in a sample tube about 1 cm above the glass insert. Cap the sample tube, heat it at 41°C for 30 min using a heating block, and analyze it. Table 1 shows the amount of standard added for each level of standard.
(c) *Sample analysis.*—Weigh the matrix and sunflower oil in a glass insert, and add the glass insert into a sample tube. Position a plug in a sample tube about 1 cm above the glass insert, and cap the sample tube. Heat it at 41°C for 30 min using a heating block, and analyze it (Table 2). The T_2 value for different concentration calibration standards is the same from experimental setting of the instrument pulse sequence ($T_2 = 149$ ms). The calibration curve was generated using the intensities (relative % units) of echo signals from each standard (y-axis) versus the amount of standard weight (x-axis). The echo signal is generated by the following phenomenon.

(d) The Hahn echo pulse program is composed of a short 90° RF pulse (first pulse, $\pi/2$ angle), wait time (3.5 ms), 180° refocus RF pulse (2nd pulse, π angle), and the same wait time (3.5 ms). When a sample is initially introduced in the instrument, the net magnetic moment vector of a sample is on the z axis along with magnetic field of the permanent magnet. A 90° short RF pulse will put the magnetization vector on transverse plane (xy plane). During the short waiting time, T_2 relaxation starts to happen and vectors will disperse (signal decays). The 180° refocus pulse will put vectors on the opposite side of equatorial plane. The same amount of wait time will refocus the vector and echo signal will be generated. The instrument measures the peak of the echo signal, and the calibration curve is generated as echo signal intensity to the standard concentrations.
(e) *System suitability.*—The instrument suitability is checked each day before use. Daily check sample (rapeseed) is purchased from the vendor, and is used to check the power supply, receiver, modulator, transmitter, and magnet on the instrument.

Calculation

$$\text{Total fat} = \frac{\text{Fat(g)}}{\text{Sample(g)}}$$

where total fat = amount of fat per gram of sample; fat (g) = fat amount determined by the instrument; and sample (g) = total weight of a sample.

Results and Discussion

Single-Laboratory Validation (SLV) Parameters

This method validation work was conducted following the guidelines of AOAC INTERNATIONAL SLV criteria (15).

Linearity/Range

Generate a curve by plotting signal intensities of 10 different standard concentrations against their concentrations.

(a) *Acceptance criterion.*—The coefficient of determination (R^2) of the linear curve must be ≥0.999.
(b) *Result.*—The results yield a coefficient of determination (R^2) of 1.0000, and it meets the acceptance criterion. Table 3

Table 1. Calibration curve standard weights

Fat, %	Standard weight, g
125	1.5003
117	1.4035
108	1.3094
100	1.2007
92	1.1007
83	1.0030
63	0.7545
42	0.5036
21	0.2550
0	0.0000

Table 2. TD-NMR parameters

Scan number	16
Dummy scan	2
Receiver gain	49
Echo time, τE	7 ms
Integration width	0.1 ms
Recycle delay (RD)	1.5 s

Table 3. Signals obtained for fat standards

Standard weight, g	Signal intensity
1.5003	67.580
1.4035	63.114
1.3094	58.911
1.2007	54.053
1.1007	49.483
1.0030	45.087
0.7545	33.870
0.5036	22.636
0.2550	11.510
0.0000	0.022

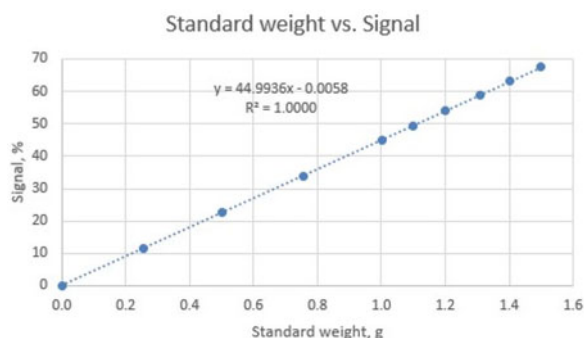shows the signal obtained for each standard concentrations, and Figure 2 shows the linearity of the curve .

Figure 2. Linearity/range result

Specificity

Prepare the following two samples and analyze them: 2.5139 g matrix; 1.3169 g matrix spiked with 1.2057 g sunflower oil.

(a) *Acceptance criterion.*—Not more than 0.1% fat should be detected on the matrix sample.
(b) *Result.*—0.0% (0.001 g) fat was detected on the matrix sample, and 1.201 g fat was detected on the spiked matrix sample. The specificity results meet the acceptance criterion.

Precision

Prepare six replicate samples at 100% level and analyze them.

(a) *Acceptance criterion.*—The RSD of the six test results should not be more than (NMT) 2%.
(b) *Result.*—The RSD of the six recovery results is 0.3%, which meets the acceptance criterion. Table 4 summarizes the test results.

Table 4. Precision result

Name	Matrix, g	Sunflower oil, g	Result, g	Recovery, %	Average	RSD, %
Sample 1	1.3169	1.2057	1.201	99.6	99.8	0.3
Sample 2	1.3323	1.2018	1.196	99.5		
Sample 3	1.3242	1.2016	1.197	99.6		
Sample 4	1.3096	1.2027	1.205	100.2		
Sample 5	1.3112	1.2073	1.208	100.1		
Sample 6	1.3292	1.2084	1.206	99.8		

Table 5. Accuracy result

Name	Placebo, g	Sunflower oil, g	Sample #	Result, recovered, g	Recovery, %
80%	1.5439	0.9561	1	0.959	100.3
			2	0.959	100.3
			3	0.960	100.4
100%	1.3041	1.2121	1	1.213	100.1
			2	1.213	100.1
			3	1.214	100.2
120%	1.0643	1.4400	1	1.434	99.6
			2	1.435	99.7
			3	1.435	99.7

Table 6. Ruggedness result between two analysts on different days

Name	Placebo, g	Sunflower oil, g	Result, recovered, g	Recovery, %
Analyst 1, #1	1.3169	1.2057	1.201	99.6
Analyst 1, #2	1.3323	1.2018	1.196	99.5
Analyst 1, #3	1.3242	1.2016	1.197	99.6
Analyst 1, #4	1.3096	1.2027	1.205	100.2
Analyst 1, #5	1.3112	1.2073	1.208	100.1
Analyst 1, #6	1.3292	1.2084	1.206	99.8
Analyst 2, #1	1.3055	1.2040	1.200	99.7
Analyst 2, #2	1.3023	1.2113	1.212	100.1
Analyst 2, #3	1.3046	1.2012	1.205	100.3
Analyst 2, #4	1.3032	1.2005	1.207	100.5
Analyst 2, #5	1.3029	1.2028	1.211	100.7
Analyst 2, #6	1.3092	1.2004	1.210	100.8
			Average	100.1
			RSD, %	**0.4**

Accuracy

Prepare three different concentration of samples (80, 100, and 120%), and analyze them in triplicate. Calculate the recovered amount of total fat in percentage recovery.

(a) *Acceptance criterion.*—All spiked recoveries should be within 98–102%.

(b) *Result.*—Table 5 shows the recovery results for triplicate sample preparation at each concentration of three different concentrations of samples. All recovery results ranging from 99.6 to 100.4% meet the acceptance criterion.

Ruggedness

The second analyst prepared six replicate samples at the 100% level and these samples were analyzed on a different day. Total fat results of the second analyst are tabulated with those of the first analyst and the overall RSD from both analysts was calculated.

(a) *Acceptance criterion.*—The RSD of 12 test results from two chemists should be not more than (NMT) 5%.

(b) *Result.*—Table 6 shows the RSD of 12 test results from both analysts is 0.4%. The ruggedness result has met the acceptance criterion.

Conclusions

The validation results obtained for linearity/range, specificity, precision, accuracy, and ruggedness demonstrated that the TD-NMR test method for the determination of total fat in sunflower oil powder is suitable for its intended use.

Funding

Conflict of Interest

None declared.

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