Nuclear Magnetic Resonance (NMR) Spectroscopy applied to the analysis of Unsaturated Fat content in Seed Oils

Application Note – Pulsar 002

Analysis of 60 MHz ^{1}H Spectrum of Seed Oil

5 samples of 10 different seed oils were dissolved in chloroform in a 1:1 ratio by volume. 1 mL of each sample was transferred to 5 mm NMR tubes. A 60 MHz ^{1}H NMR spectrum was acquired for each of the samples. The area under the resonances A, C and D was estimated by peak integration. The value of integral A was set to 4 corresponding to 4 ^{1}H nuclei per glycerol backbone and the other integrals were calculated as a ratio with respect to this value. Providing estimates for the average number of olefinic (C) and bis-allylic ^{1}H nuclei per triglyceride molecule. The results were averaged across the five samples for each oil and are summarised in table 1 along with the results for similar samples of pure triglycerides, glyceryl tristearate, glyceryl trioleate and glyceryl trilinoleate described previously.

The complete spectra can also be analysed using principal component analysis (PCA). The principal components can then be used as classifiers to distinguish between different oils. Figure 7 shows the plot of the two most significant principal components and illustrated the separation between the different oils. While it is convenient to illustrate this classification with a two dimensional plot, statistically more than two principal components can be used as discriminators.

Table 1: *Average number of olefinic and bis-allyllic ^{1}H nuclei estimated by peak integration. The colours in brackets after the name of the seed oil is the colour used to represent the data for each oil in figure 7.*

Seed Oil	Olefinic (^{1}H per triglyceride)	Bis-allyllic (^{1}H per triglyceride)
glyceryl tristearate (grey)	-	-
glyceryl trioleate (grey)	6.17 ± 0.07	-
glyceryl trilinoleate (grey)	12.1 ± 0.1	5.9 ± 0.2
Rapeseed (canola, blue)	8.4 ± 0.1	1.4 ± 0.1
Sunflower (black)	9.9 ± 0.1	2.6 ± 0.1
Olive (green)	6.08 ± 0.05	0.29 ± 0.04
Corn (maize, red)	9.9 ± 0.2	2.68 ± 0.09
Peanut (ground nut,purple)	7.0 ± 0.1	0.96 ± 0.04
Rice bran (orange)	7.5 ± 0.2	1.5 ± 0.2
Walnut (magenta)	11.5 ± 0.3	5.0 ± 0.2
Linseed (brown)	12.5 ± 0.2	6.6 ± 0.2
Coconut (cyan)	0.31 ± 0.05	-
Palm (pink)	3.58 ± 0.06	0.35 ± 0.02

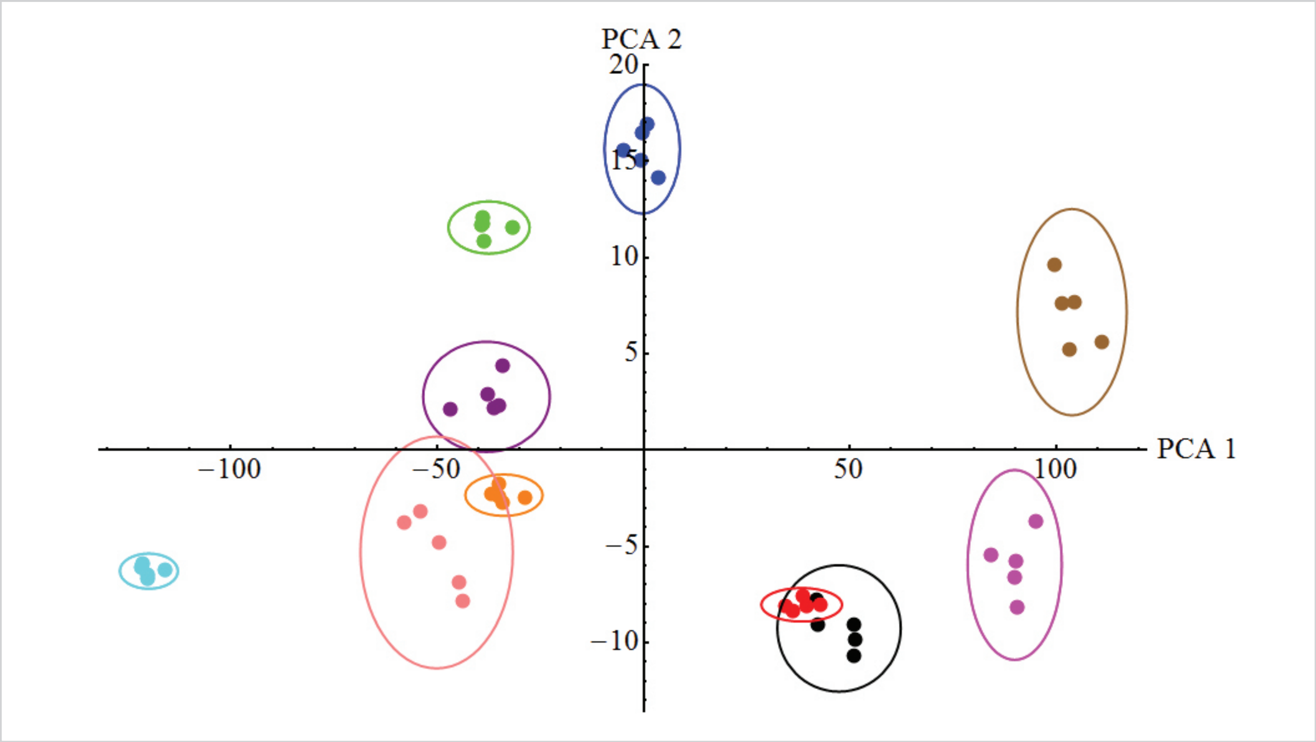

Figure 7: *Classification of seed oils using principal component analysis of the full spectrum.*

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Background

The constituents of plant derived oil fall under the general class of chemicals defined as lipids. Prof. William Christie classifies them as "fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds". With further clarification of the term fatty acid as "compounds synthesised in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex. They usually contain even numbers of carbon atoms in straight chains (commonly C_{14} to C_{24}), and may be saturated or unsaturated; they can also contain other substituent groups" [1]. See figure 1 below. Although a variety of lipids and starch comprise the storage tissues of many food plants the major constituents of oil seeds are the triacylglycerol lipids; three fatty acid chains attached as esters to a glycerol back bone. The fatty acid composition of oils is commercially important, e.g., high oleic acid content, as found in olive oils, has been implicated in a reduced risk of heart disease and linoleic acid, found in sunflower oils, is an essential nutritional fatty acid. High levels of these fatty acids can increase the commercial value of an oil whereas excessive amount of linolenic acid can decrease the value since it is more susceptible to oxidation and problems with rancidity.

While most plant oils are produced for food and feed, up to 15% of some oils, e.g., soy and rapeseed, and 100% of certain commodity oils, e.g., castor and tung, have non-food related industrial applications. Most oils comprise five primary fatty acids, palmitic, stearic, oleic, linoleic and linolenic that can be utilised in the production of lubricants, surfactants, polymers and inks as well as their used in food/feed applications. The fatty acids of commodity oils tend to have little or no nutrition value, however their unusual chemical properties make them ideal for particular industrial applications. The most well known example is the laurate fatty acid derived from coconut oil which has excellent foaming properties that makes it useful in the production of anionic surfactants.

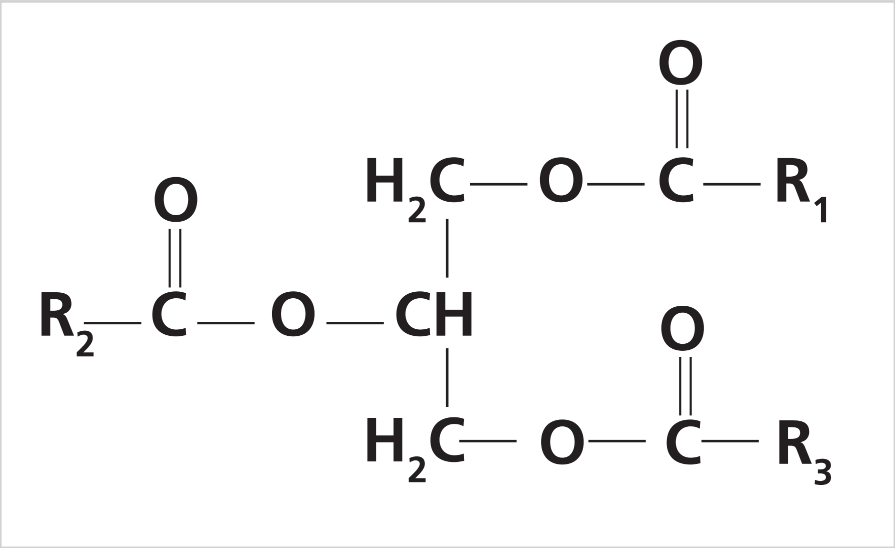

Figure 1: *Schematic representation of a typical triacylglycerol. R_1, R_2 and R_3 are aliphatic chains that may or may not contain double bonds.*

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Glycerolipids

The class of substances described as lipids covers a broad range of chemicals including fats, waxes, sterols and the fat soluble vitamins whose primary functions include energy storage, cell membrane structure and biochemical signalling. Industrially the most common lipids are the glycerolipids. The glycerolipids are mainly composed of mono, di and tri-substituted glycerol (shown in figure 2). Of these the triacylglycerols, also referred to as triglycerides, are by far the most common.

Triacylglycerols

In the triacylglycerols (TAG) each of the three hydroxyl groups of the glycerol backbone are esterified with a fatty acid. Typically the TAG will contain three different fatty acids. There are many different types of TAG with their level of saturation being dependent on the constituent fatty acid chains. Figure 3 shows a triglyceride comprising three different fatty acid chains; a saturated C_{16} chain fatty acid (palmitic), a monounsaturated C_{18} chain (oleic) and a polyunsaturated C_{18} chain fatty acid (linonelic). Different seed oils have different levels of the fatty acid chains and this composition determines their physical and chemical properties, which in turn determines their commercial use.

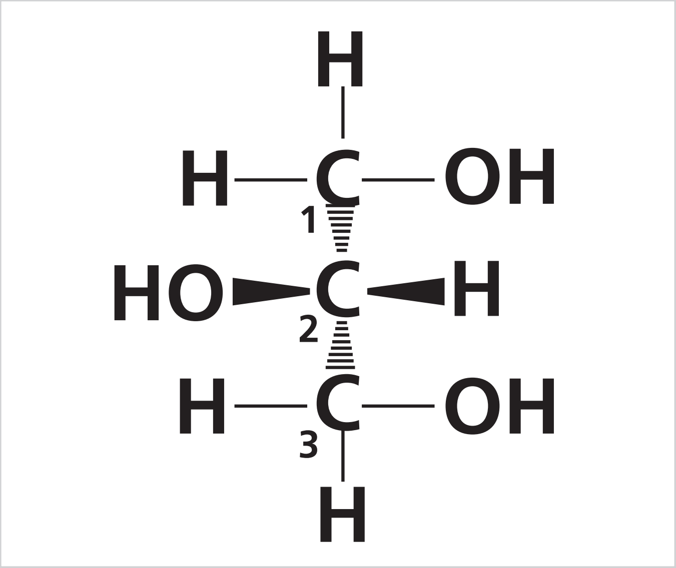


Figure 2: *Chemical structure of glycerol*

The different chemical groups give rise to specific signals in an NMR spectrum, which at high field, typically 9.4 T, provide a characteristic fingerprint or fatty acid profile that can be used to identify an oil [2]. In this report it is demonstrated that different chemical groups are still clearly identifiable in the ^1H NMR spectrum even at lower field, i.e., 1.4 T corresponding to a proton Larmor frequency of 60 MHz.

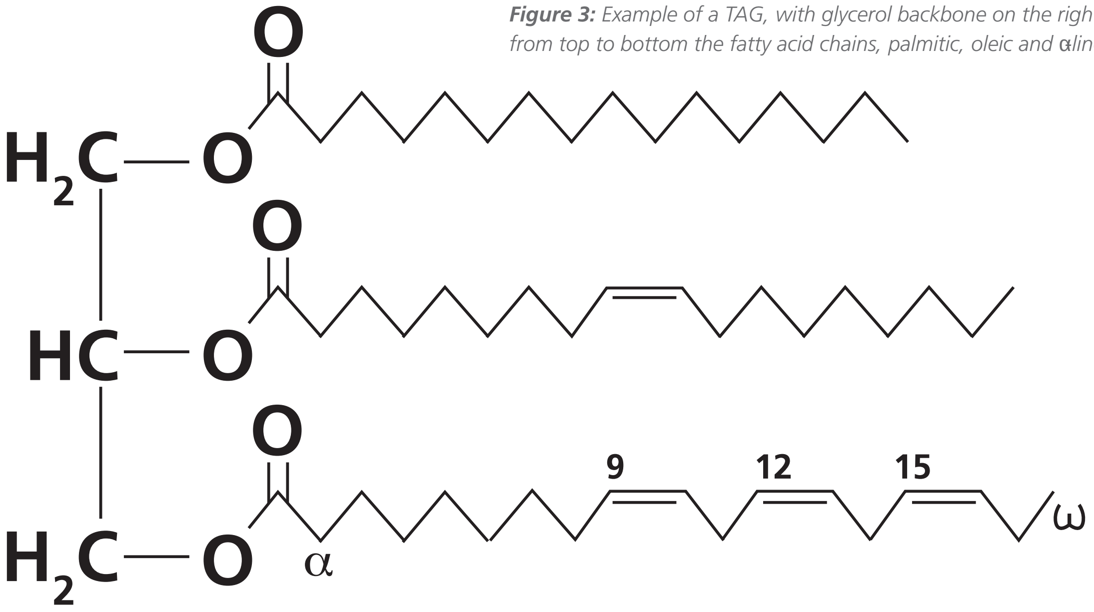


Figure 3: *Example of a TAG, with glycerol backbone on the right and from top to bottom the fatty acid chains, palmitic, oleic and αlinolenic.*

Analysis

Anatomy of the 60 MHz, ^1H Spectrum of Triacylglycerols

The ^1H spectrum of glyceryl trilineolate (figure 4), a triglyceride where all the fatty acid chains derive from linoleic acid, is shown in figure 5. It is possible to identify various resonances with specific chemical groups in the molecule. Resonance A originates from the four ^1H nuclei attached to carbon 1 and 3 on the glycerol backbone. This is a particularly useful signal because it is a measure of the number of glyceride molecules in the sample and as such can be used as a reference signal. The signal from the fifth ^1H nuclei lies at about 5.3 ppm, directly below signal C. This must be taken into account when integrating the peaks.

Resonance C can be assigned to the ^1H nuclei attached to carbons with a double bond, usually referred to as olefinic. This peak is a measure of the total level of unsaturation in a triglyceride, regardless of whether it is in a monounsaturated or polyunsaturated chain. Resonance D originates from a methylene group ($-CH_2-$) sandwiched between two double bonds, referred to chemically as bis-allyllic. This is specifically a measure of polyunsaturation.

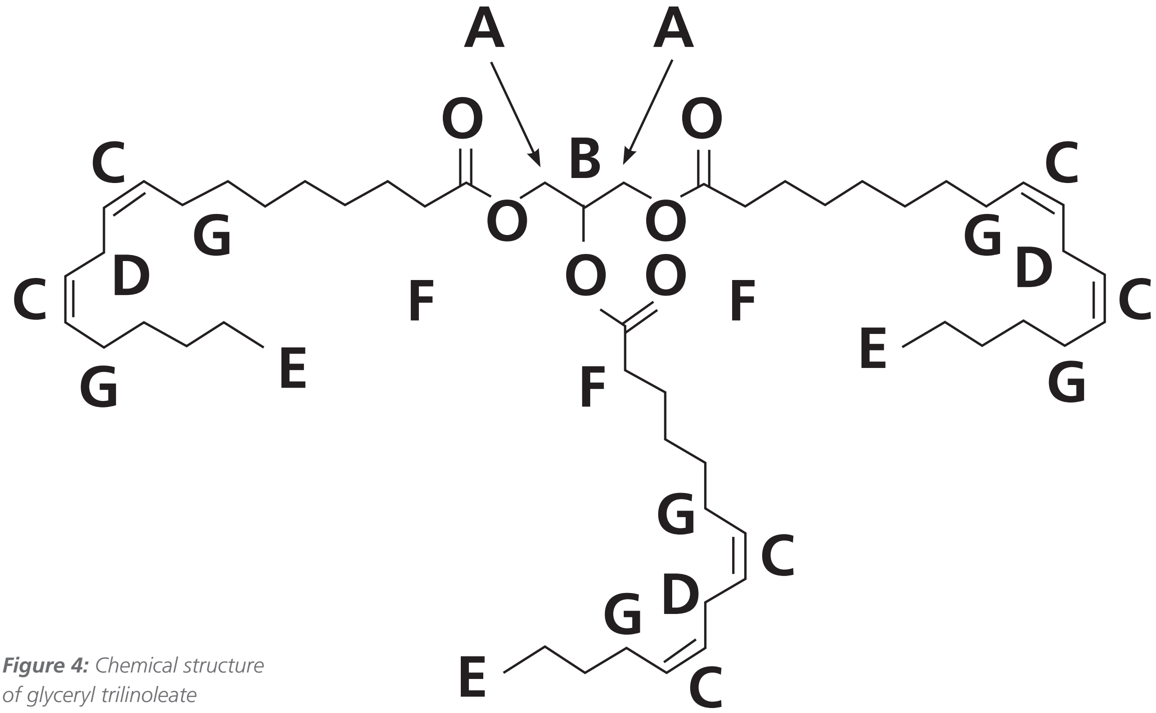


Figure 4: Chemical structure of glyceryl trilinoleate

Resonance E can be assigned to the terminal methyl group ($-CH_3$) for each chain. Resonance F comes from the methylene group on the fatty acid chain closest to the ester coupling with the glycerol back bone and resonance G comes from a methylene group with a neighbouring double bond, often referred to chemically as an allylic group.

The large peak at approximately 1.2 ppm originates from the remaining methylene groups in the fatty acid chains. The integral of resonances A, C and D can be used to quantify the level of unsaturation in a seed oil. The ratio of integral C and A gives the average number of olefinic ^1H nuclei per triglyceride and as such is a measure of overall unsaturated character. The ratio of integral D and A provides an estimate of the number of bis-allyllic ^1H nuclei, which is a measure of polyunsaturation

To illustrate these estimates the spectra of glyceryl trilinoleate (bottom), glyceryl trioleate (middle) and glyceryl tristearate (top) are shown in figure 6. As shown in figure 4 glyceryl trilinoleate is comprised of a glyceryl backbone with three linoleic fatty acid chains. The spectrum shows both a large olefinic resonance and a bis-allyllic resonance.

Glyceryl trioleate comprises a glycerol backbone with three oleic acid chains. Oleic acid has a single double bond in each chain and as can be seen from the spectrum, there is a significant olefinic resonances, but no bis-allyllic resonance. The allyllic resonance is clear in the spectrum of both glyceryl trilinoleate and glyceryl trioleate.

Glyceryl tristearate comprises a glycerol backbone with three stearic acid chains. Stearic acid is a fully saturated C_{18} chain and, as can be seen from the spectrum there is no bis-allyllic signal, no allyllic signal. While there appears to be a rather small olefinic resonance at ~5.3 ppm, this is in fact the signal from the ^{1}H nuclei attached to carbon 2 on the glycerol backbone.

The integral ratios are shown in table 1 and it can be seen that these are commensurate with the unsaturated character of the molecule

Figure 5: *^{1}H spectrum of glyceryl trilinoleate obtained at 60 MHz.*

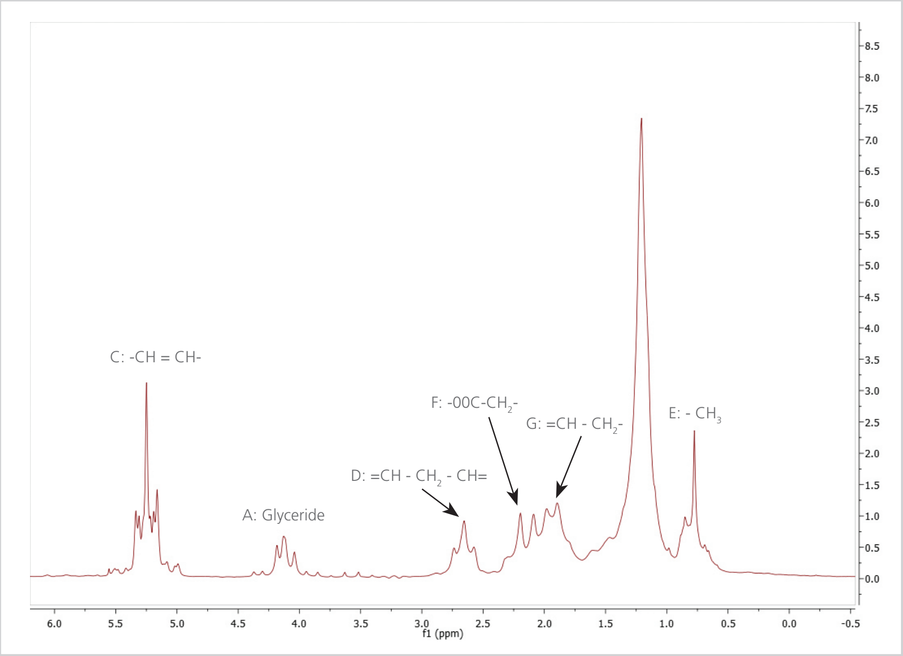
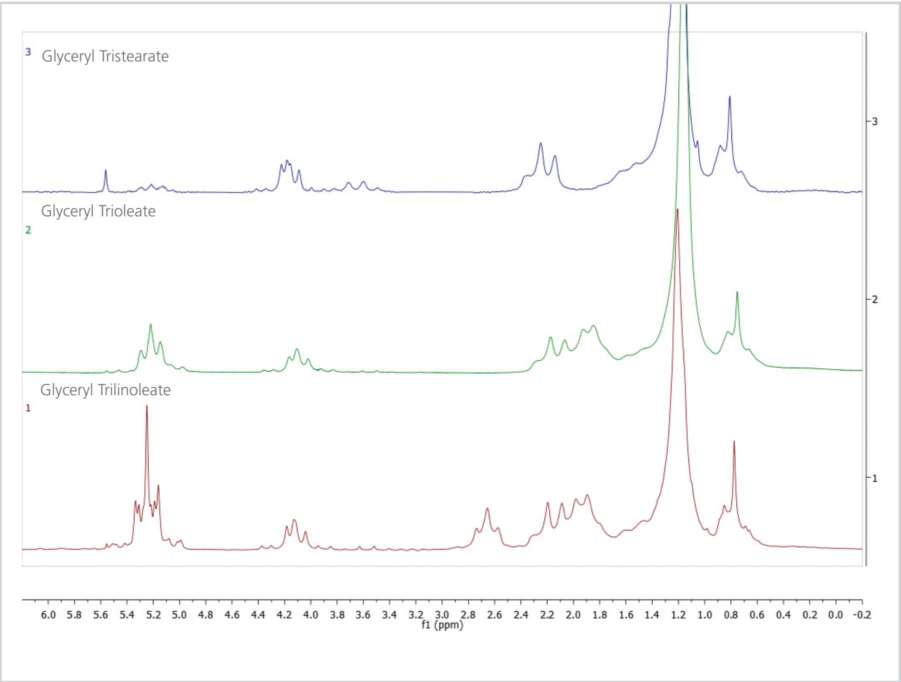


Figure 6: *Comparison of the 60MHz ^{1}H spectrum of glyceryl tristearate, glyceryl trioleate and glyceryl trilinoleate*



Summary

In conclusion, 60MHz NMR spectroscopy provides a suitable tool for the study of seed oils. The chemical specificity of the spectral peaks can be used as markers to estimate the levels different types of the fatty acid chains. The analysis of the spectra could be extended using a line fitting package. This could be used to estimate signal amplitudes from overlapping resonances, especially those associated with the methylene and methyl groups. The integral of this region of the spectrum provides information about the average fatty acid chain length.

The analysis of the whole spectrum using chemometric methods such as principal component analysis provides a suitable framework for classification of different types of oil. This work could be extended beyond simple classification to provide a method for the detection of adulterants or monitoring seasonal changes in oil crops.

[1] http://lipidlibrary.aocs.org/Lipids/whatlip/index.htm

[2] Guillen M. D. and Ruiz A. Eur. J. Lipid Sci Technol.
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