

Cholesterol

Cholesterol belongs to the group of sterol lipids. It is biosynthesized by all animal cells and is essential for the structure of cell membranes. It also functions as precursor for the biosynthesis of steroid hormones and vitamin D. Figure 1 shows the ^1H NMR spectrum of 250 mM Cholesterol in CDCl_3 measured in a single scan taking 10 seconds to acquire.

1D Proton spectrum

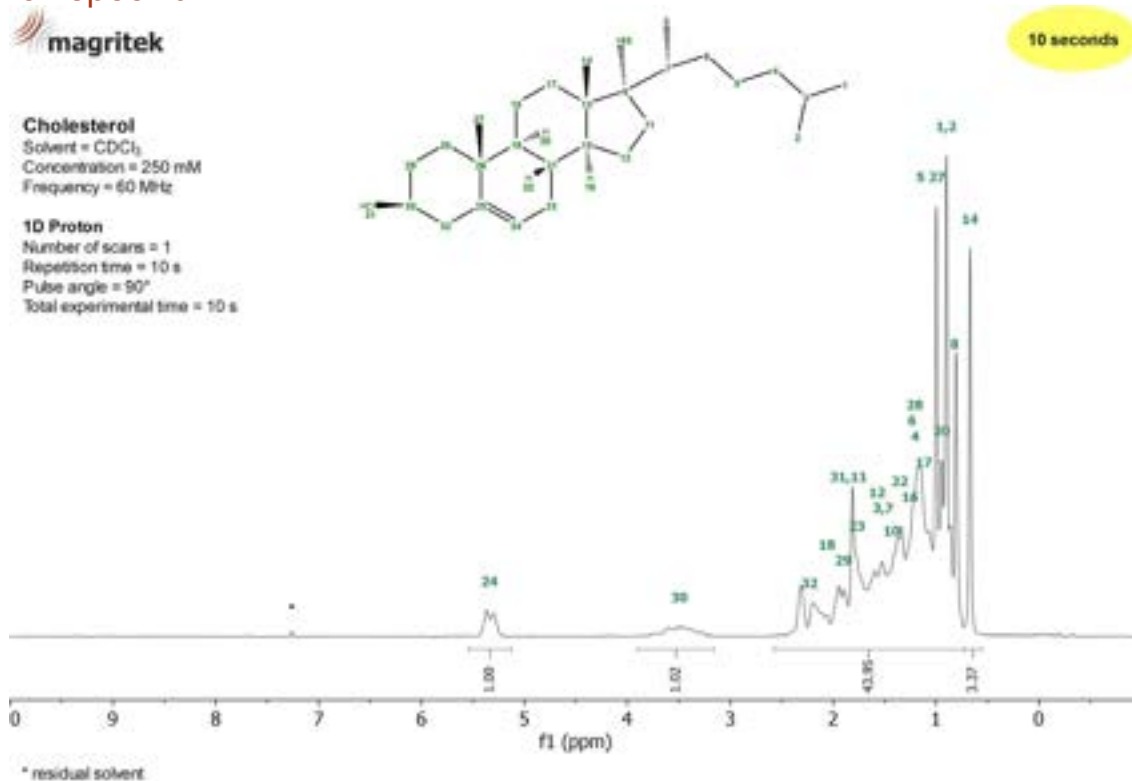


Figure 1: ^1H NMR spectrum of 250 mM Cholesterol in CDCl_3 measured on a Spinsolve 60 MHz system in a single scan.

1D Carbon spectrum

Figure 2 shows the ^{13}C NMR spectrum of 250 mM Cholesterol in CDCl_3 acquired using NOE polarization transfer from ^1H to ^{13}C and ^1H decoupling. The 1D Carbon experiment using NOE is sensitive to all ^{13}C nuclei in the sample. It clearly resolves all the expected resonances.

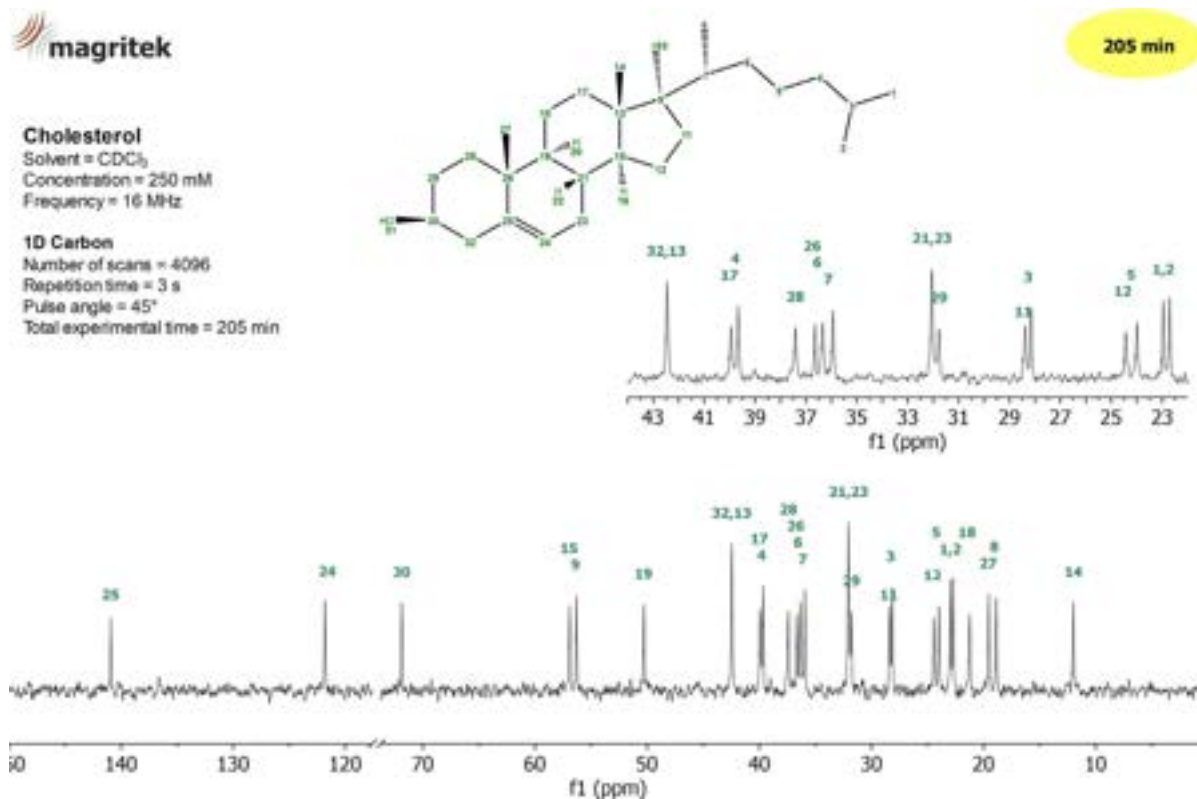


Figure 2: ^{13}C NMR spectrum of 250 mM Cholesterol in CDCl_3 measured on a Spinsolve 60 MHz system in 205 minutes.

2D COSY spectrum

The 2D COSY experiment allows one to identify coupled ¹H nuclei as they generate cross peaks out of the diagonal of the 2D data set. In Figure 3 a large number of cross peaks can be nicely observed. For example, the proton at position 24 couples to protons 23 and 32 (pink), the proton 30 couples to protons 29 (light green) and protons 32 (orange).

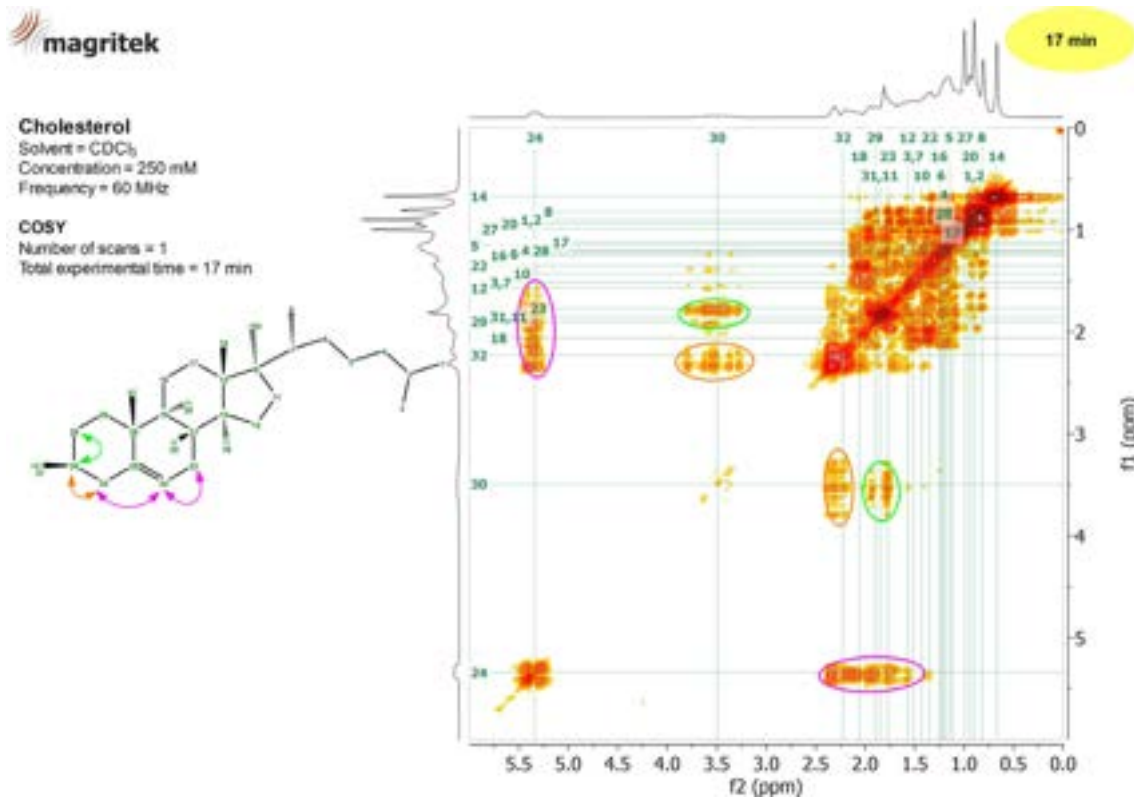


Figure 3: ¹H 2D COSY experiment of 250 mM Cholesterol in CDCl₃ acquired in 17 minutes on a Spinsolve 60 MHz system.

2D HSQC-ME

The HSQC is a powerful sequence widely used to correlate ¹H with the one-bond coupled ¹³C nuclei. The Spinsolve is equipped with a multiplicity edited version (HSQC-ME) of this method. It provides the editing power of the DEPT-135 sequence, which is useful to differentiate the signals of CH₂ groups (blue) from CH and CH₃ groups (red). Figure 4 shows the HSQC-ME spectrum of 250 mM Cholesterol in CDCl₃ acquired in 34 minutes.

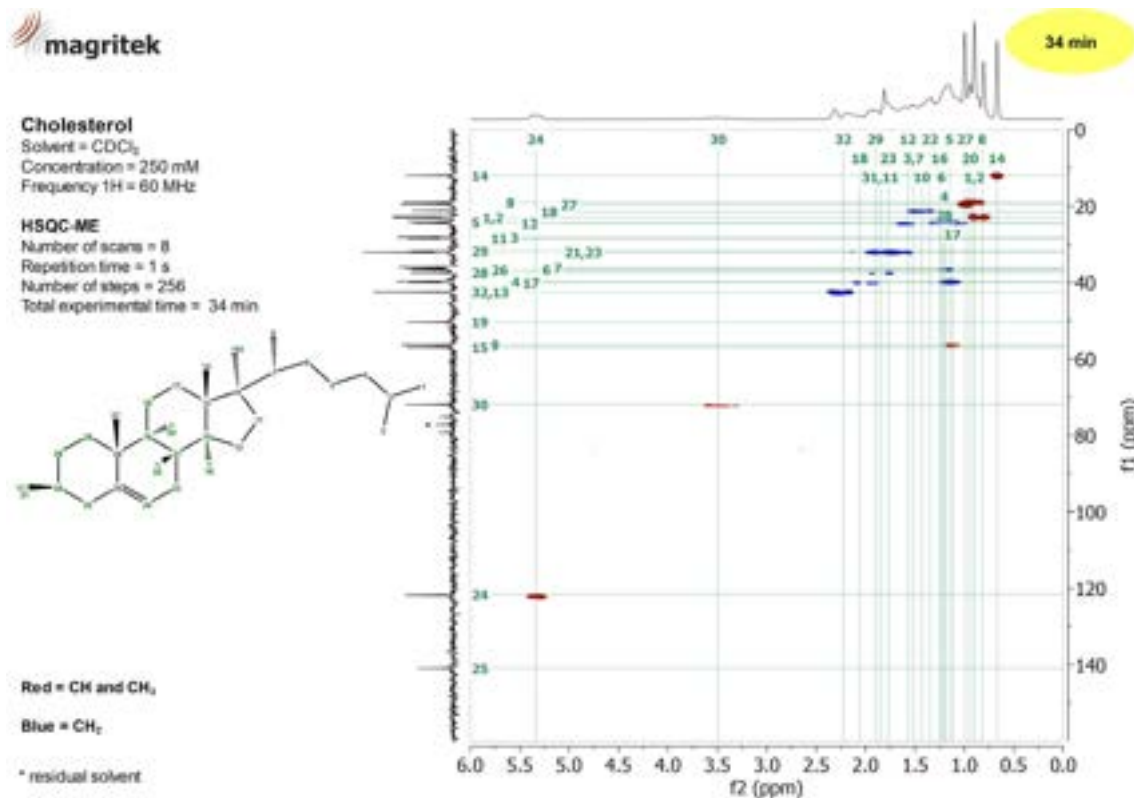


Figure 4: HSQC-ME spectrum of a 250 mM Cholesterol sample in CDCl₃ showing the correlation between the ¹H (horizontal) and ¹³C (vertical) signals.

2D HMBC

To obtain long-range ¹H-¹³C correlations through two or three bond couplings, the Heteronuclear Multiple Bond Correlation (HMBC) experiment can be used. Figure 5 shows the HMBC spectrum of a 250 mM Cholesterol sample measured in 69 minutes on our Spinsolve 60 MHz. As an example, the long-range correlations of proton 32 with carbons 29 (dark pink), 30 (pink), 24 (light blue) and 25 (orange) are marked with circles. The experiment shows the correlation with quaternary carbons, too.

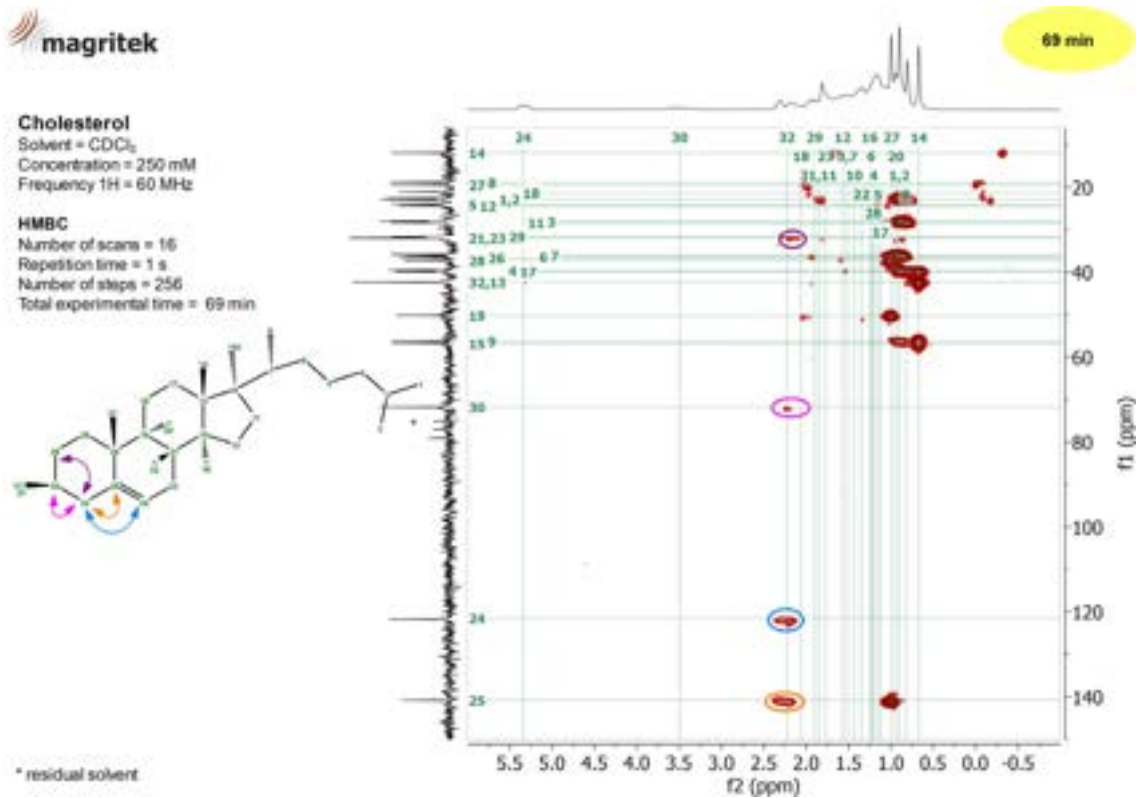


Figure 5: HMBC spectrum of a 250 mM Cholesterol sample in CDCl₃, showing the long-range couplings between ¹H and ¹³C nuclei.

Contact **Sep Sci** for more information.

Separation Scientific

Tel: 011 794 4117 | Email: Info@sepsci.co.za | Web: www.sepsci.co.za