

Absolute Content Determination by Quantitative NMR (qNMR) Spectroscopy: A Curious Case of Aldosterone

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[1] Calculation of Absolute Content of Aldosterone S1 and S2:

The following formula was utilized to calculate the absolute content of Aldosterone (from commercial source S1 and S2):

$$Px = \frac{Ix * Nstd * Mx * mstd}{Istd * Nx * Mstdmx} * Pstd$$

Px : Purity of the analyte (Aldosterone) as mass fraction; $Pstd$: Purity of the internal qNMR standard as mass fraction; Ix : Integral of the analyte (Aldosterone); $Istd$: Integral of the internal standard; Nx : No. of analyte (Aldosterone) protons; $Nstd$: No. of internal qNMR standard protons; Mx : Molecular weight of the analyte (Aldosterone); $Mstd$: Molecular weight of the qNMR internal standard; mx : Mass of analyte (Aldosterone); $mstd$: Mass of qNMR internal standard

The following values are common to all the qHNMR measurements:

Molecular Weight of Aldosterone = 360.444 g/mol; Molecular weight of the qNMR internal standard ISTD (Tecnazene = 260.890 g/mol; Purity of the qNMR internal standard as mass fraction = 99.82%; Number of Protons taken into account, for both the ISTD and Aldosterone = 1 each; Integral of Aldosterone proton = 1 (alkene proton).

Table 1 : qNMR calculation of the absolute content of S1 and S2 Aldosterone, with aldosterone quantification signal set to an integral area value of 1.

Aldosterone source	Measurement No.	Mass of Aldosterone weighed (mx)	Mass of qNMR ISTD weighed (mstd)	Integral of the qNMR ISTD (Istd)	Absolute Content of Aldosterone (Px) %
S1	1	3.9088	1.9430	0.7301	93.91
S1	2	4.8432	2.5879	0.7876	93.56
S1	3	2.5978	1.3418	0.7584	93.93
S2	4	1.7510	1.6436	1.5816	81.85

[2] 1D and 2D-NMR spectra of S1 and S2 Aldosterone utilized for quantification and analyses purpose:

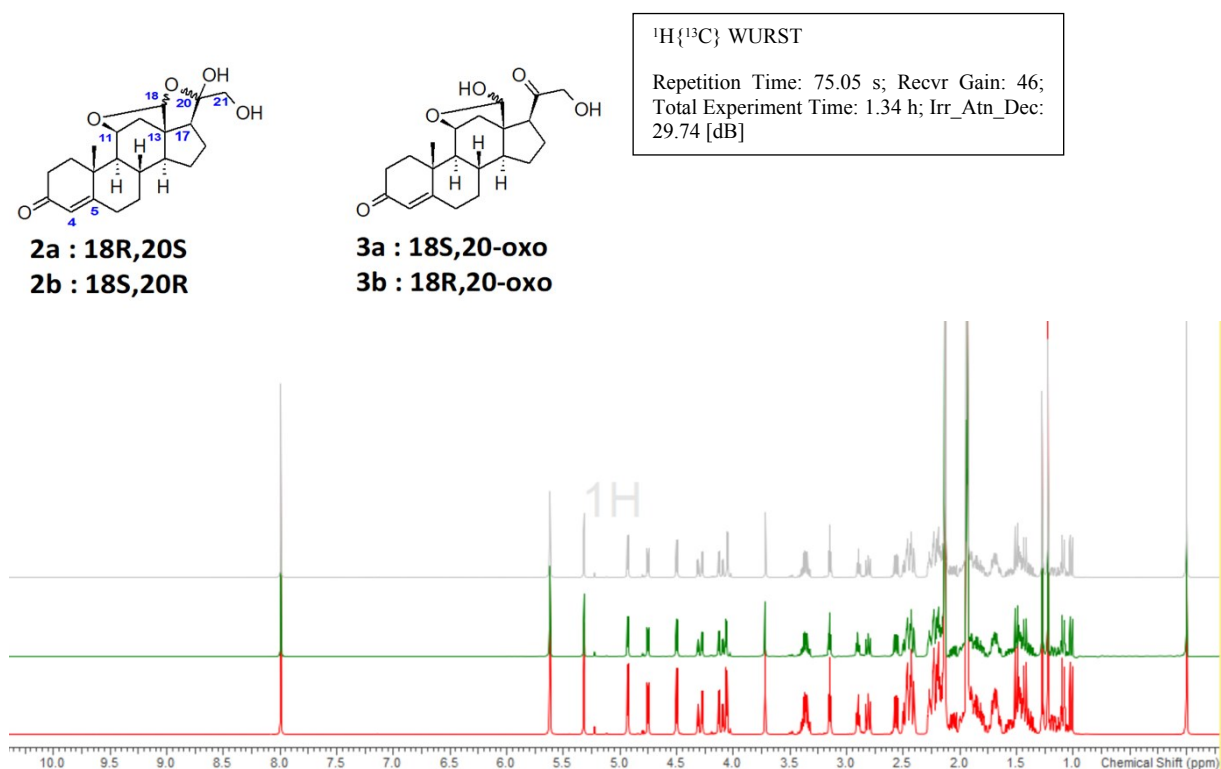


Figure 1. Overlay of the ^1H NMR spectra for Aldosterone S1

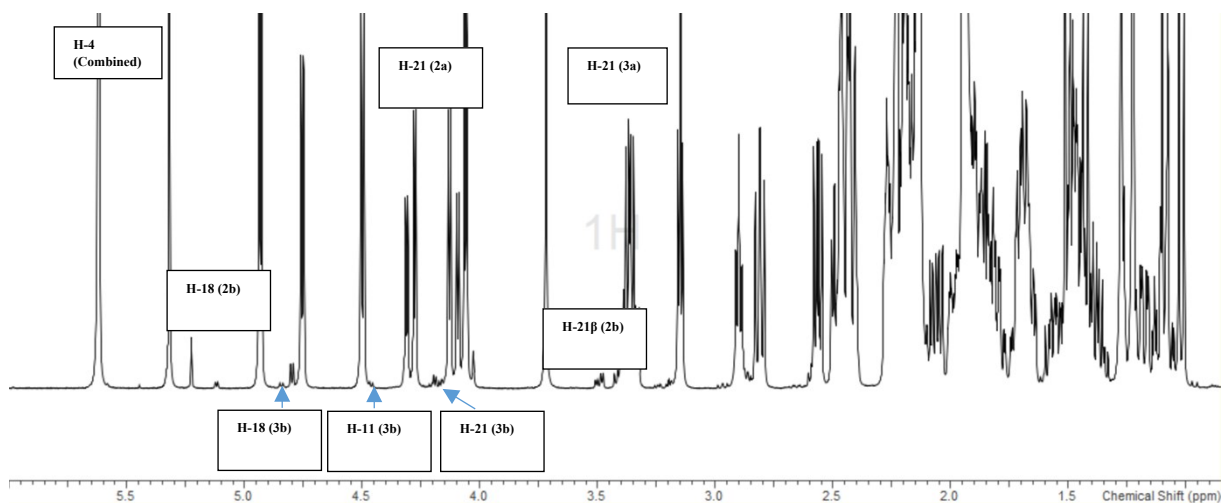


Figure 2. Expansion of the ^1H NMR spectrum of Aldosterone S1 (Experiment 1).

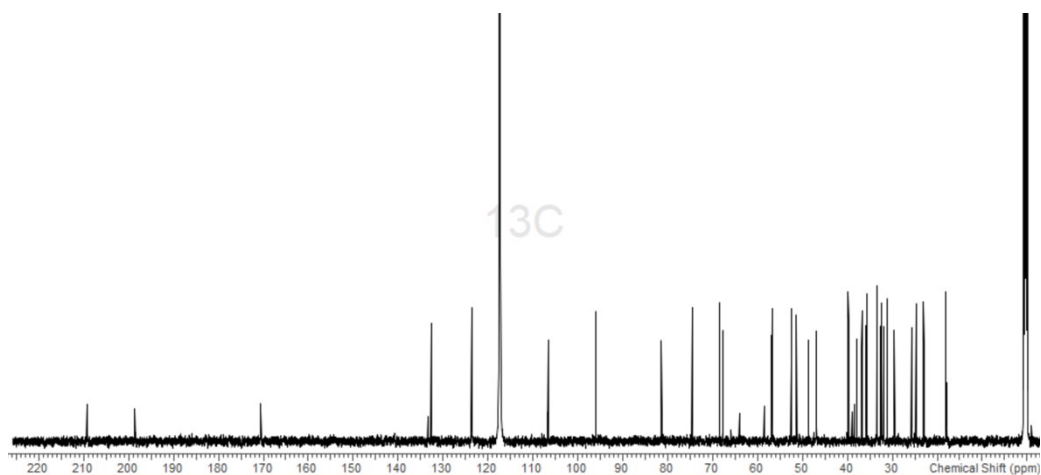


Figure 3. ^{13}C NMR of Aldosterone 1 (Experiment 1)

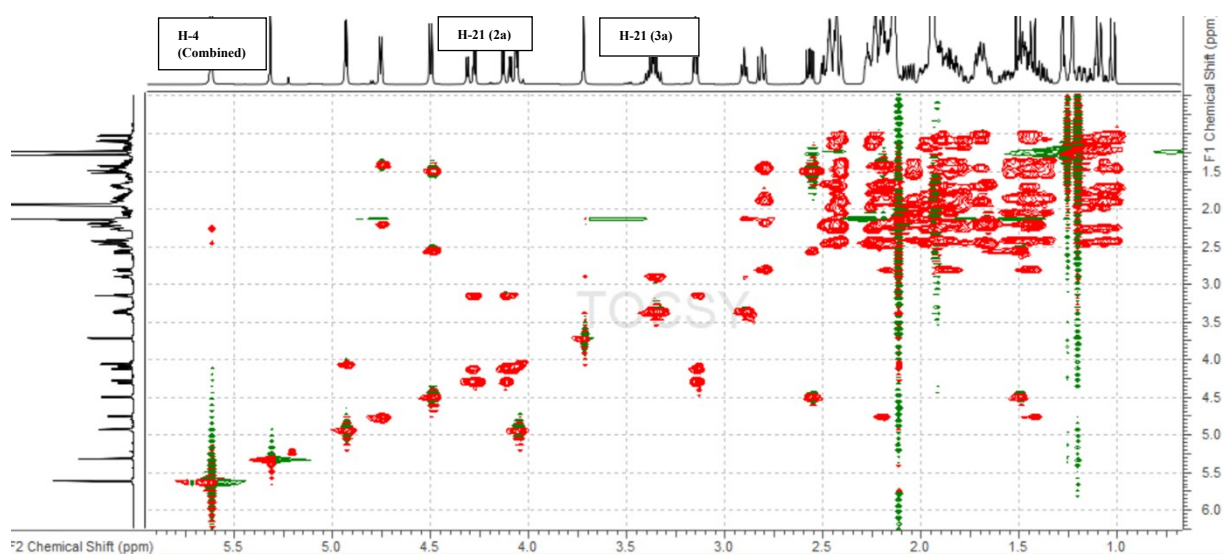


Figure 4. 2D-TOCSY spectrum of Aldosterone S1 (Experiment 1)

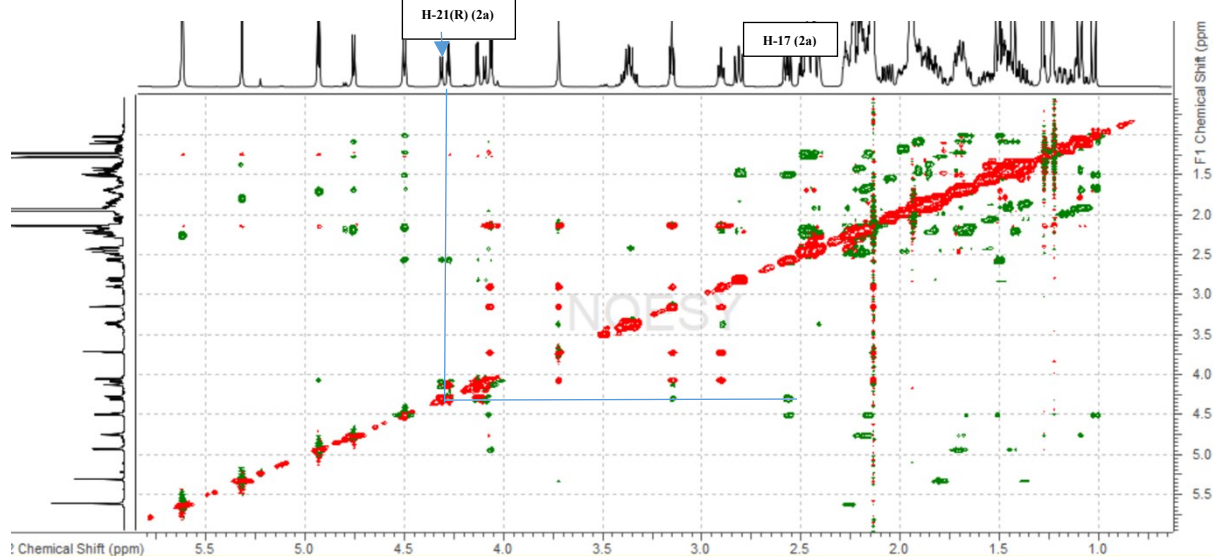


Figure 5. 2D-NOESY of Aldosterone S1 (Experiment 1).

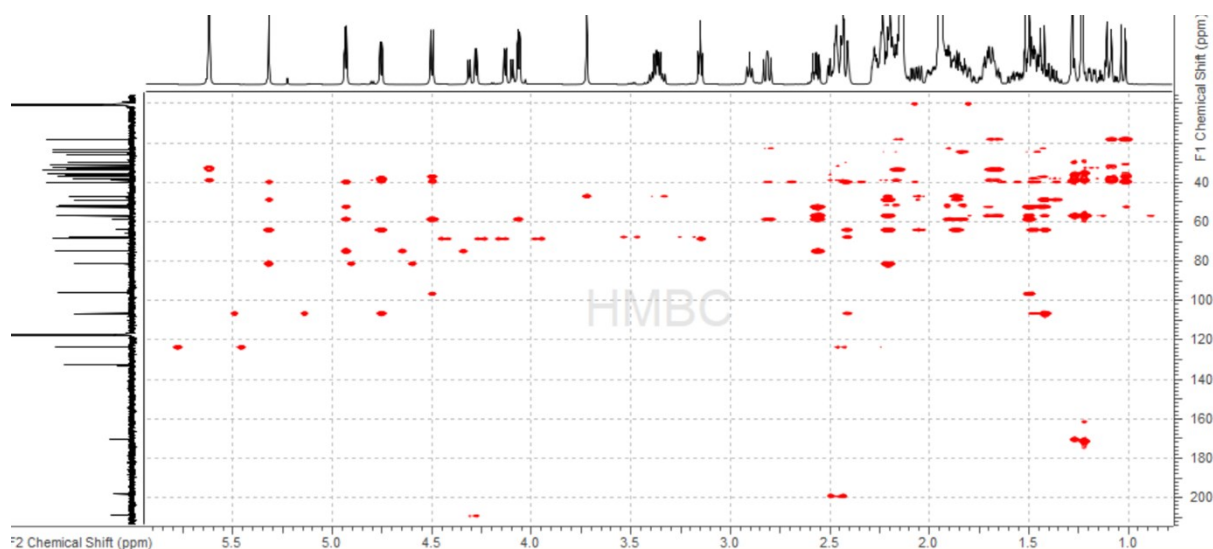


Figure 6. 2D-gHMBCAD (optimized for 3 Hz) of Aldosterone S1 (Experiment 1).

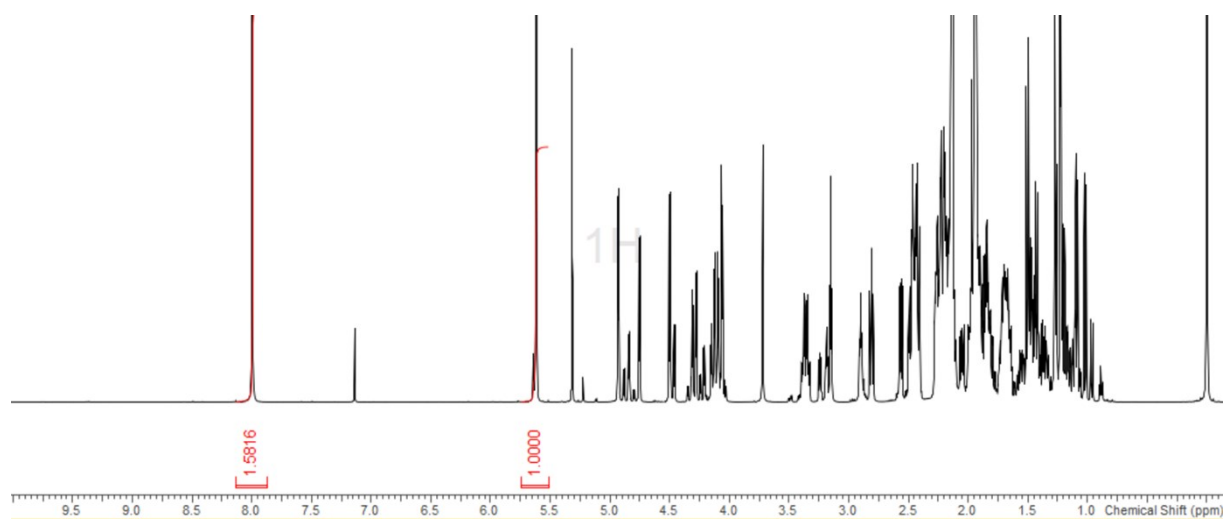


Figure 7. ^1H NMR spectrum of Aldosterone S2, including qNMR internal standard

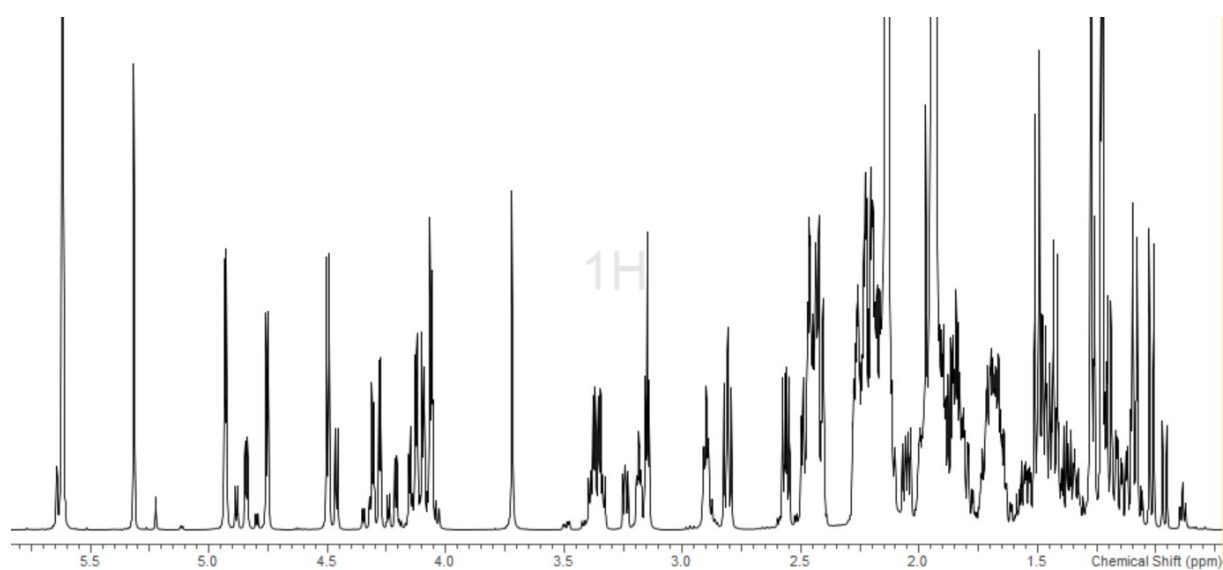


Figure 8. Expansion of the ^1H NMR spectrum of Aldosterone S2.

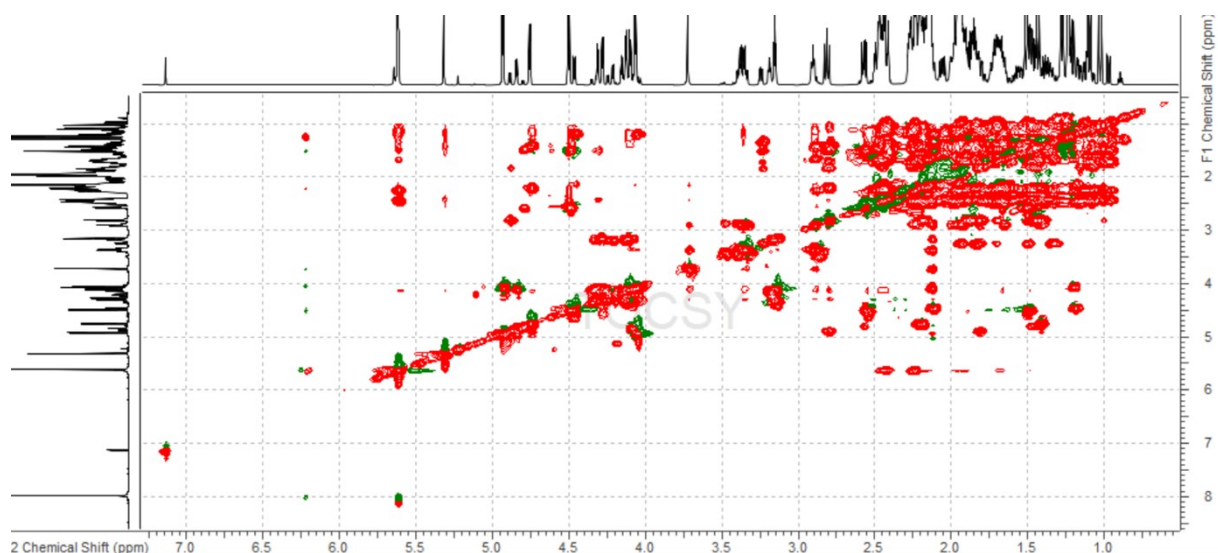


Figure 9. 2D-TOCSY of Aldosterone S2.

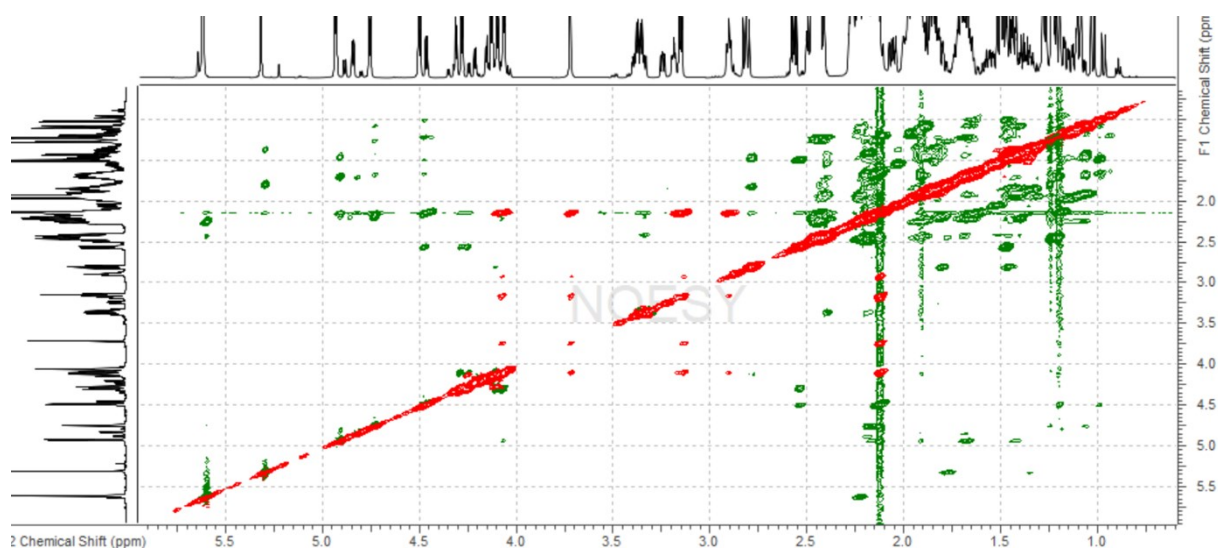
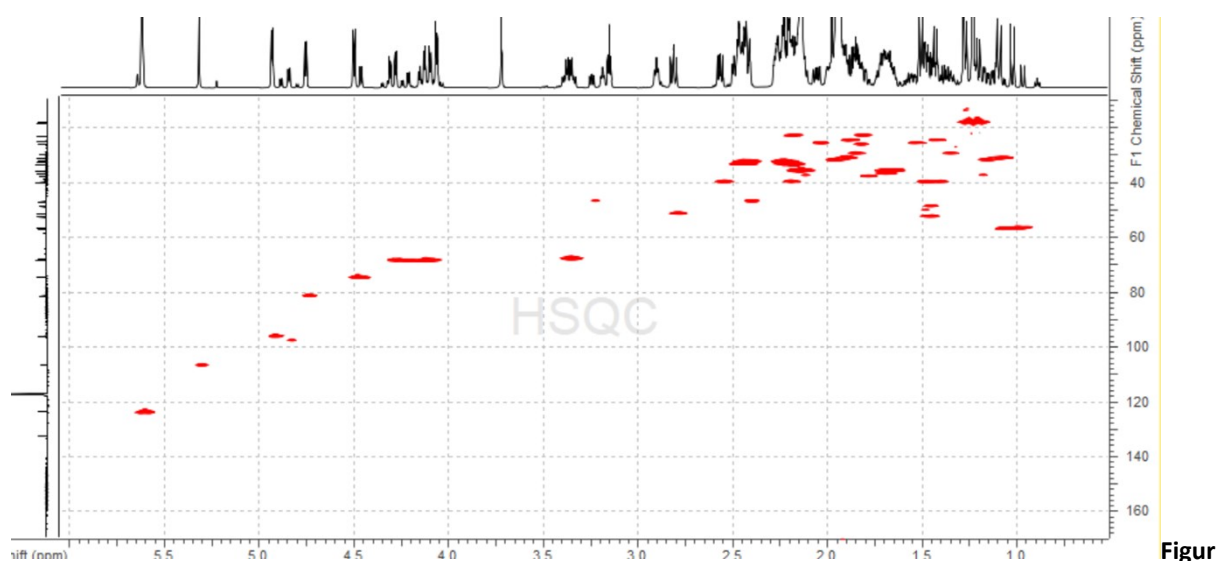


Figure 10. 2D-NOESY spectrum of Aldosterone S2.



e 11. 2D-HSQC of Aldosterone S2.

Figure