Identification

1 Description

Q Sepharose Big Beads is a macroporous, strong anion exchanger with quaternary ammonium groups coupled to the matrix (Sepharose Big Beads) which consists of agarose highly cross-linked with epichlorohydrin. Q Sepharose Big Beads are supplied as a suspension in 20% ethanol.

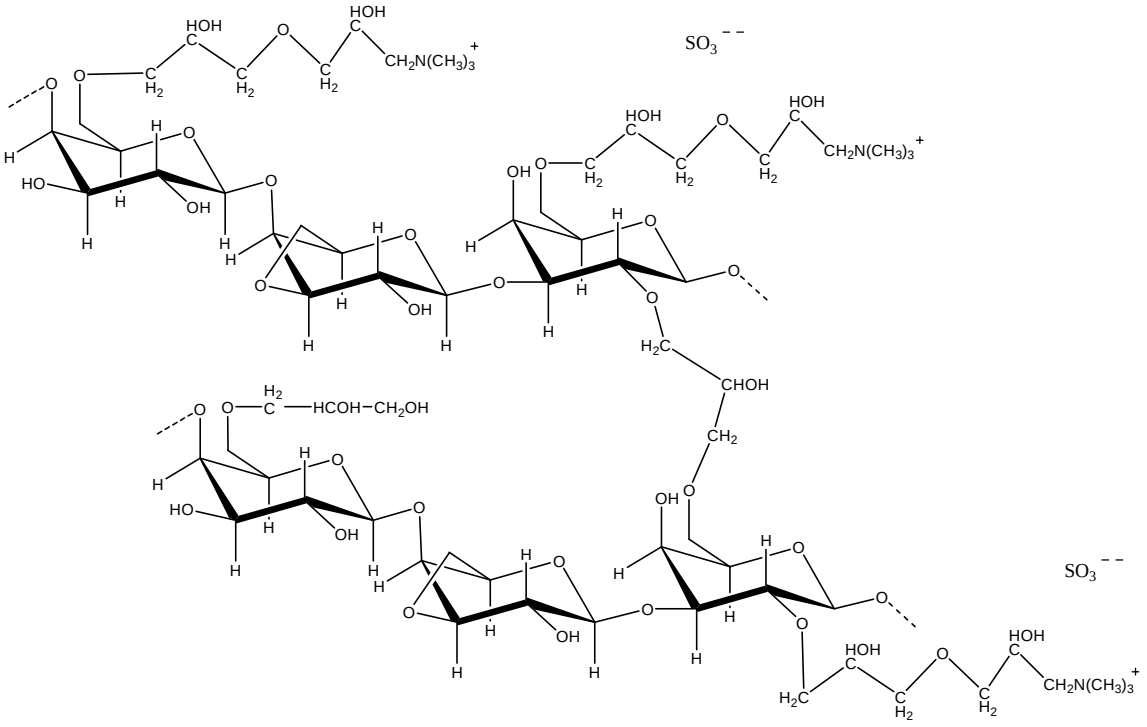

Figure 1. Structural representation of a fragment of Q Sepharose Big Beads.

The representation of complex gel structures with chemical formula is not possible owing to the fact that the detailed structure has not been elucidated and also the fact that the substitution can take place at many different hydroxyl groups.

The molecular weight of this insoluble gel has not been determined. Any attempt to solubilize the gel would lead to degradation.

2 Characterization

2.1 Nitrogen analysis

The experimentally determined value for Q Sepharose Big Beads is 2.2%.

The theoretical value for the structure presented in Figure 1 is 1.97%.

2.2 Sulphate analysis

Sulphate, present as the counter ion, has been determined by elemental analysis of sulfur. The mean value is 2.05%.

The theoretical value for the structure presented in Figure 1 is 2.25%

2.3 FTIR-PAS Spectroscopy

Infrared spectroscopy was performed using Photo acoustic spectroscopy (PAS) detection at a resolution of 8 cm^{-1} and 400 scans. All spectra have been max-min normalised. The details of the method (taken for a similar product) are given in Figure 2. The assignment of the peaks is described in Table 1. The spectra are presented in Figures 3-6.

Pharmacia Biotech

Vibrational Spectroscopy

FTIR-PAS

Page 1 of (1)
Method no: 45-2002-24
Edition: AA
Valid from: 1997-04-24
Supersedes: None

Issued by:	Approved by:
Karl-Gustav Knuuttila Analytical Method Development R&D	Bo-Lennart Johansson Analytical Method Development R&D

Product: **SP Sepharose® Big Beads, 17-0657-00**

Fourier Transform Infrared Photoacoustic Spectroscopy, FTIR-PAS
FTIR-PAS[1] directly measures the absorbance spectrum of a dry material with a minimum of sample handling. The PAS spectrum is normalized and background corrected when ratioed to a spectrum of Carbon Black.

Test parameters
The photoacoustic detector MTEC M 200 mounts directly in the sample compartment of a Perkin-Elmer 2000
FTIR spectrometer. The photoacoustic spectrum is measured with the following parameters set:

Mirror velocity (OPD) = 0.2 cm/s	Resolution = 8 cm^{-1}
Phase correction = magnitude	Interferogram type = uni-direct, double-sided
Spectral range = 4000 - 450 cm^{-1}	Apodization = Norton-Beer strong

Detector gas atmosphere = helium; the cell compartment is purged with dry helium for at least 30 s.

Performance
A sample of 5 mL sedimented gel is washed with 1.0 M NaCl followed by pure water, 3 x 15 mL of each, on a sintered glass filter. Suck the gel dry for at least 5 min. Dry in vacuum at 40 to 50 °C to less than 10 millibar, preferably over night. Collect the photoacoustic spectrum of 0.1 g of dried gel. Ratioed against a Carbon Black reference (MTEC) spectrum directly gives an absorbance spectrum compatible with those acquired with conventional IR sampling techniques. Some fluctuation of the relative signal amplitude over the spectral range may occur between different preparations. Band saturation is generally more pronounced in photoacoustic spectra. The identity should therefore be based mainly on peak positions if the PAS spectrum is compared with spectra collected with other IR techniques. Remaining moisture in the sample will impair the accuracy. Desiccant placed in the cell beneath the sample cup and prolonged purging will help.

Figure 2. Test method details for FTIR-PAS.

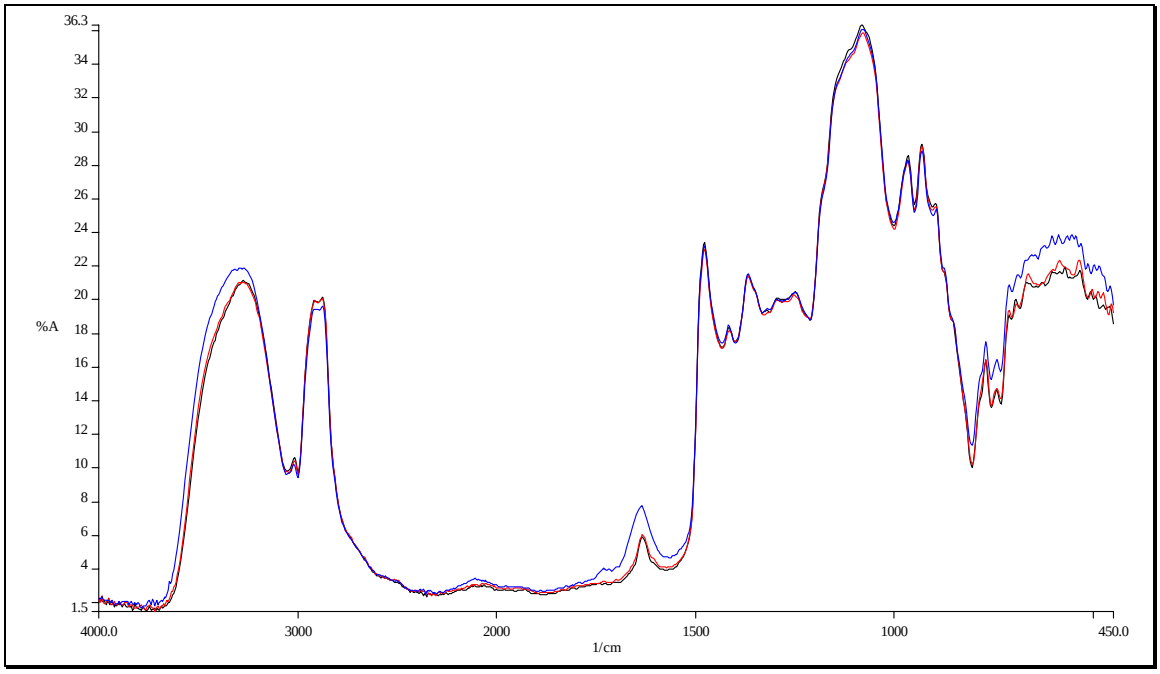

Figure 3. FTIR-PAS spectra of the three analysed batches of Q Sepharose Big Beads. Blue: T-302192, Red: T-301462, Black: T-301065.

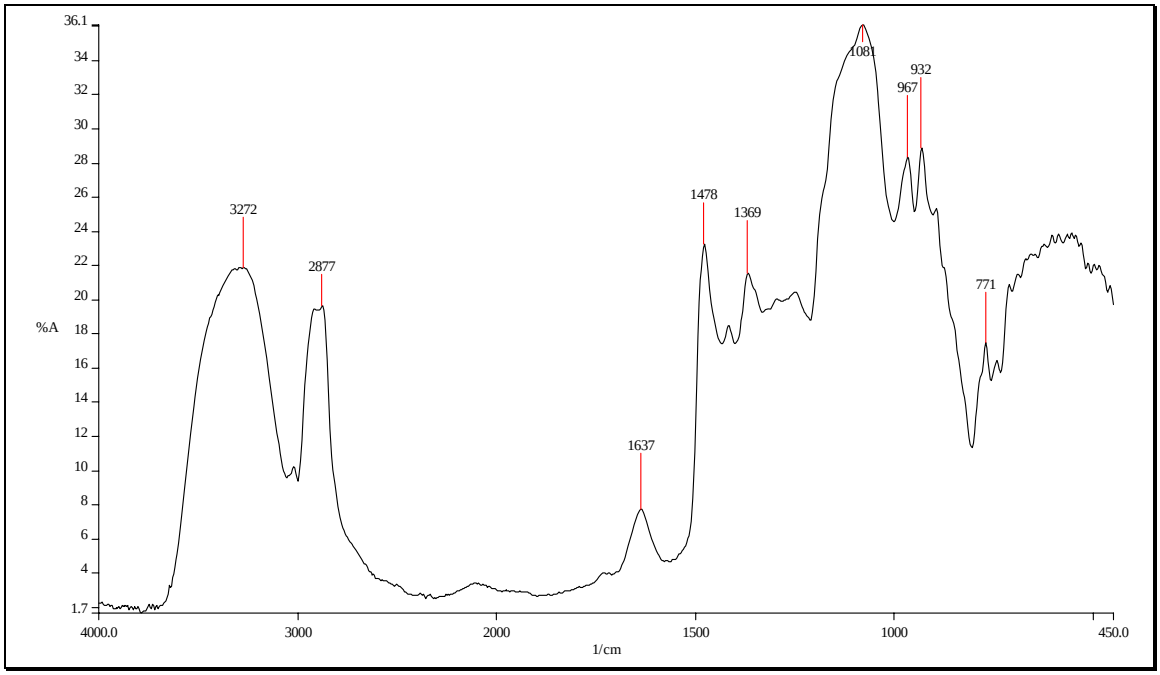

Figure 4. FTIR-PAS spectrum of batch number T-302192.

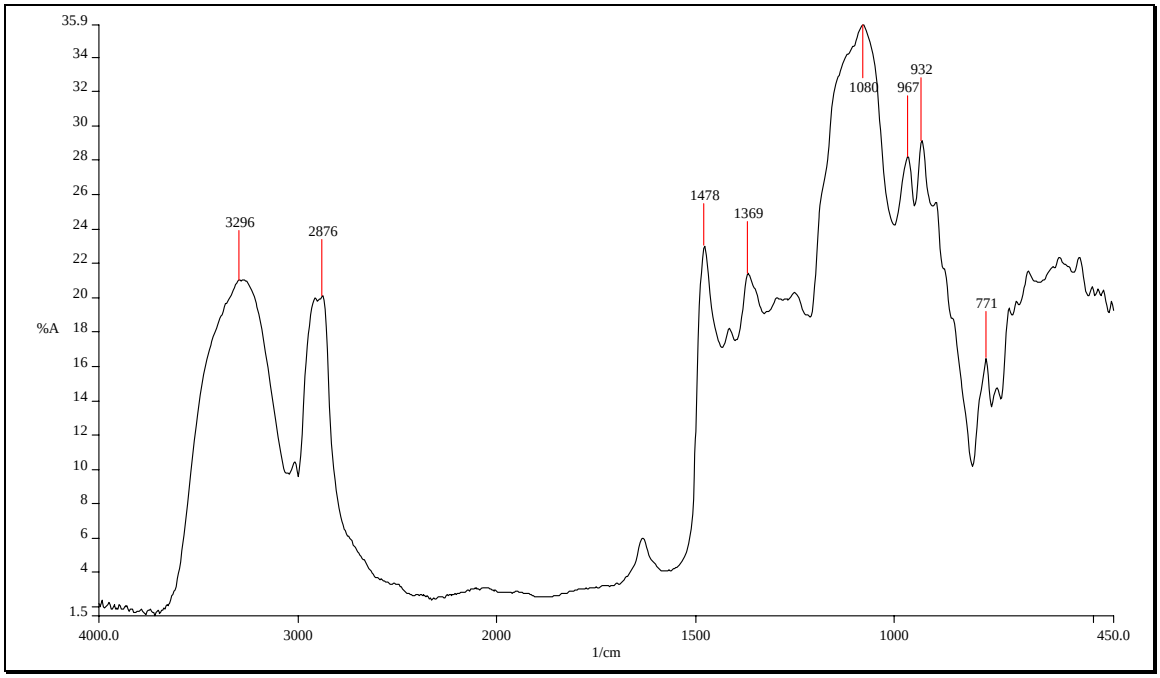

Figure 5. FTIR-PAS spectrum of batch number T-301462.

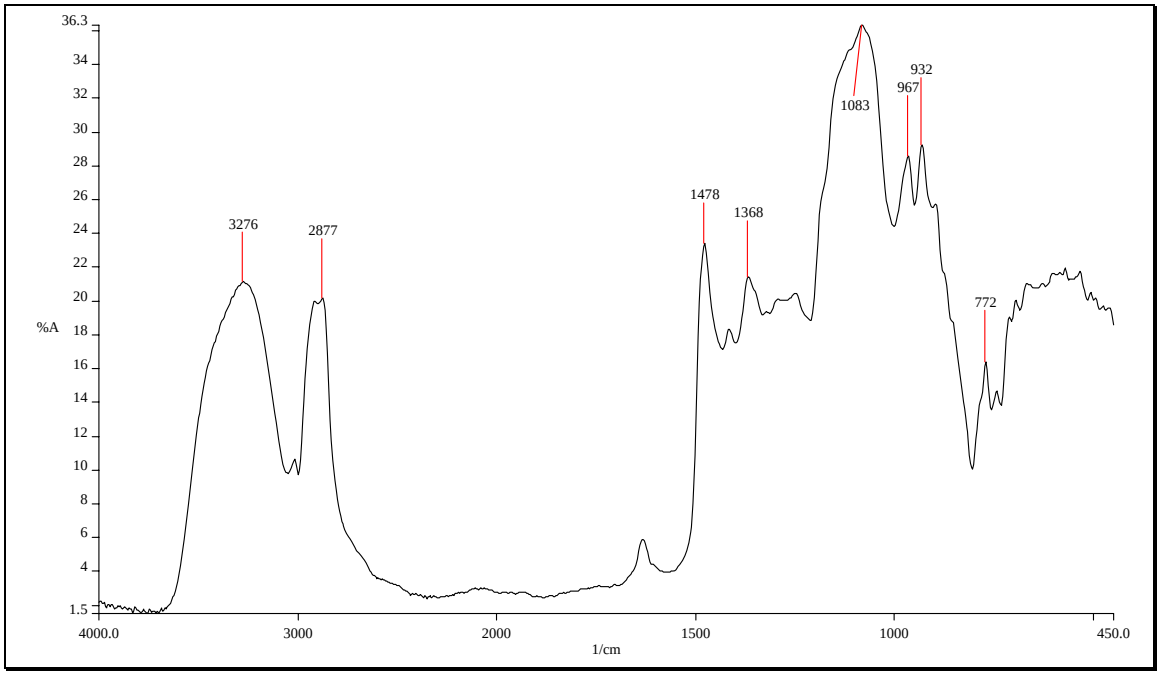

Figure 6. FTIR-PAS spectrum of batch number T-301065.

Table 1. IR assignments for Q Sepharose BB.

Wavenumber, cm^{-1}	Band assignment
3400 broad	OH stretching, H-bonded
2920,2890	CH_2 and CH stretching
1470	CH_2 bending (N^+ groups)
1150	C-O-C asymmetric stretching (glycodic linkage)
1100,1070 sh, 1040,1010	C-O stretching, C-C stretching, OH bending
850	C_1H(eq) bending

2.4 C^{13} NMR

Three batches of Q Sepharose Big Beads (T-302192, T-301462 and T-301065) were analysed with ^{13}C-NMR. The temperature was 80 °C during all the experiments. The spectra and all of the relevant experimental parameters are shown in Figures 7-9. The chemical shift was calibrated by adding a small amount of TSP-D4 (0 ppm) to one of the samples (spectrum not shown here).

The most significant feature of these spectra is the clearly distinguishable signal at 56 ppm which is ascribed to the methyl carbons on the quaternary ammonium moieties. The group of signals between 65 and 80 ppm are assigned to the ring carbons and the glycidyl carbons.

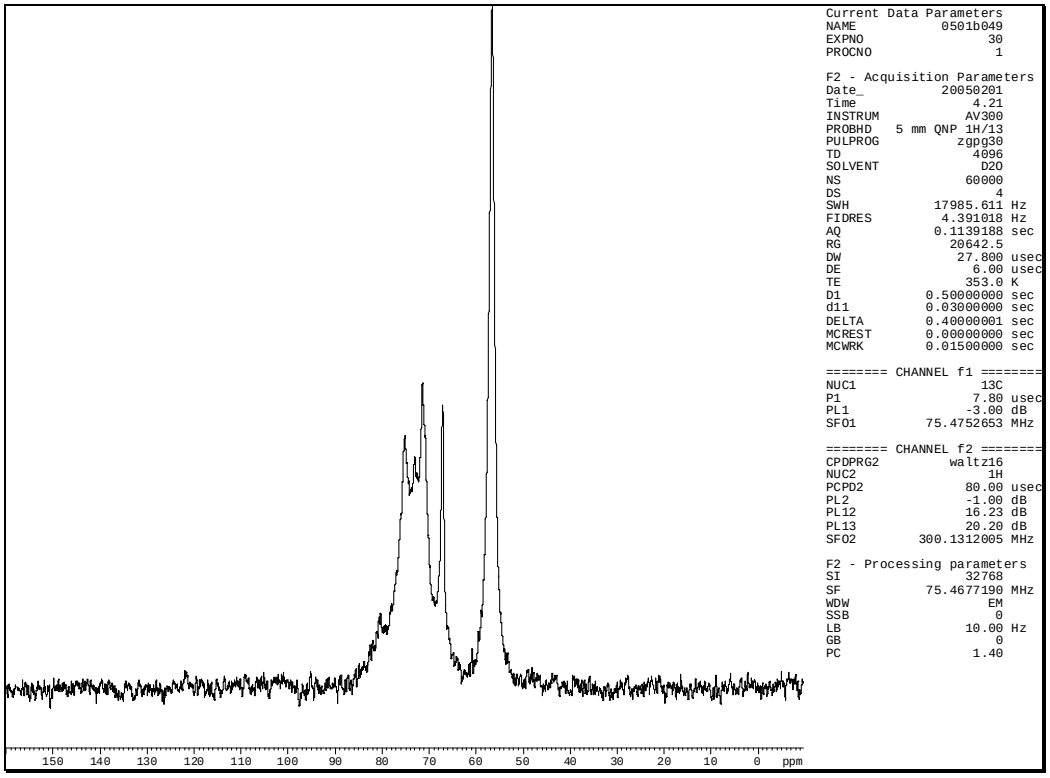

Figure 7. *^{13}C-NMR spectrum of Q Sepharose Big Beads batch T-302192.*

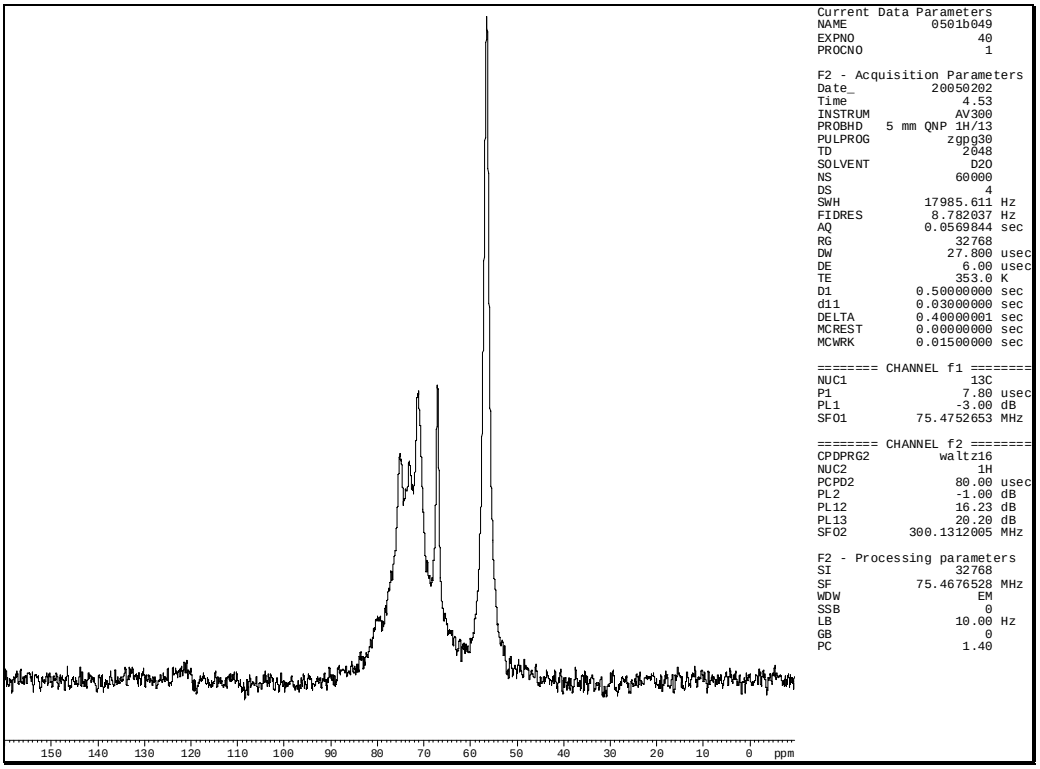

Figure 8. *^{13}C-NMR spectrum of Q Sepharose Big Beads batch T-301462.*

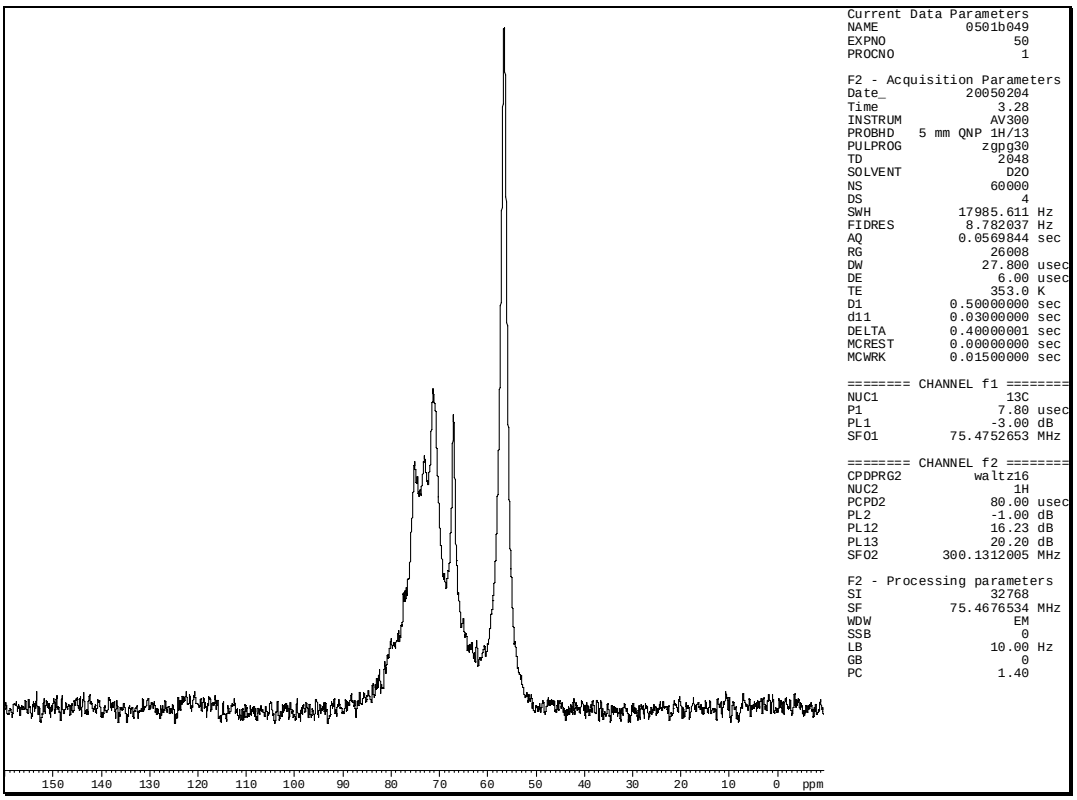

Figure 9. *^{13}C-NMR spectrum of Q Sepharose Big Beads batch T-301065. C*