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Helms, Gunther

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Technical Note:

Hetero-nuclear STEAM: Localisation of ^{13}C MR spectroscopy using $\{^{1}H$-$^{13}C\}$ polarisation transfer and 1D phase encoding

Gunther Helms

Medical Radiation Physics, Lund University, Lund, Sweden

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Corresponding author:
Gunther Helms
Lund University, Medical Radiation Physics, Dept. Clinical Sciences Lund, Barngatan 4, SE-22185 Lund, Sweden
E-mail: gunther.helms@med.lu.se

Abstract. Gradient selection of pathways makes it possible to simplify polarisation transfer sequences by omitting chemical shift refocusing pulses. The features of a simplified three pulse STEAM sequence for $\{^{1}H$-$^{13}C\}$ polarisation transfer have been studied at 2 Tesla on a whole body MR system with regard to use for in vivo MR spectroscopy. The observed signal is increased by the net polarisation of adjacent protons minus refocusing and relaxation losses. Adjustment of the J-evolution interval may be used as a J-filter to suppress unwanted resonances. A two-dimensional gradient localisation in a single acquisition was achieved by means of selective proton pulses. In combination with a one-dimensional phase encoding gradient a three-dimensional localisation of ^{13}C spectra was achieved. Depth resolution along the coil axis made it possible to separate subcutaneous from underlying signals and an external reference signal. A total measuring time of 12 minutes on human calf muscle suggests that this method may be suitable for *in vivo* studies.

Keywords: 13C spectroscopy, STEAM, polarisation transfer, gradient selection, localisation, signal enhancement

1 Introduction

MR spectroscopy (MRS) of ^{13}C provides a wealth of information on specific metabolites and metabolic rates, especially of glucose and glycogen (Gruetter et al., 2003). A major intrinsic problem of *in vivo* ^{13}C MR spectroscopy (MRS) is its low sensitivity. Thus, surface coil detection is generally regarded to be necessary (Aue et al., 1985, Beckmann and Müller, 1991). The characteristic radiofrequency (RF) field distribution of the surface coil provides a convenient localisation. Coil geometry, however, involves a trade-off between localization in the coil plane and detection of signals from deep-lying regions. In addition, unwanted signals originating from close to the coil, e.g. from subcutaneous fat, may be strongly enhanced. This makes an additional localization method desirable, e.g. using slice-selective excitation. The large chemical shift range of ^{13}C resonances (about 200 ppm), however, leads to considerable chemical shift displacement of the selected slice.

Localization and sensitivity issues may be addressed simultaneously by means of {^{1}H-^{13}C} polarisation transfer (PT) sequences. Several schemes with localisation by selective ^{1}H pulses have been reported (Norris et al., 1988, Yeung and Swanson, 1989, Watanabe et al., 1998, Kreis et al., 1997), which were based on the INEPT (Morris and Freeman, 1979) or DEPT (Doddrell et al., 1982) sequences. As these sequences are developed for high resolution MRS, they involve phase cycling and sufficiently "hard" 180° pulses, which may difficult to produce on a human MR system, even with a surface coil.

The INEPT sequence may be simplified by using gradient selection of the coherence transfer pathways (Hurd and John, 1991) instead of phase cycling. When the hard 180° pulses are omitted, signals are selected in hetero-nuclear stimulated echo acquisition mode (STEAM) (Frahm et al., 1987). By selective proton pulses, the sequence features a two-dimensional (2D) localisation in a single acquisition. As proof-of-principle, we report the implementation on a 2T whole body MR system, with 2D localisation in the coil plane and phase encoding of the ^{13}C signal along the coil axis (Beckmann and Müller, 1991), corresponding to 1D spectroscopic imaging (SI).

2 Material and methods

Experiments were performed at the MRC Clinical and Biochemical MRS Unit, Dept. of Biochemistry University of Oxford, Oxford, UK. The participating healthy human volunteers gave fully informed written consent as approved by the local ethics review board.

2.1 Equipment

The sequence was implemented on a Bruker Avance MR system (Bruker Medical, Karlsruhe, Germany) operating under the Paravision 1.0.31. software on a 2T whole body magnet (Oxford Magnet Technology, Abingdon, UK) with a maximum gradient strength of 10 mT/m. The RF hardware, a 25 cm proton birdcage resonator (85 MHz) and a 7 cm ^{13}C surface coil (21 MHz), had been developed in-house. The ^{13}C surface coil was positioned horizontally in the x-z-plane. A disk-shaped external phantom containing tetramethyl-silane (TMS) was mounted in the coil centre to calibrate the flip angle of the carbon pulse and as chemical shift reference.

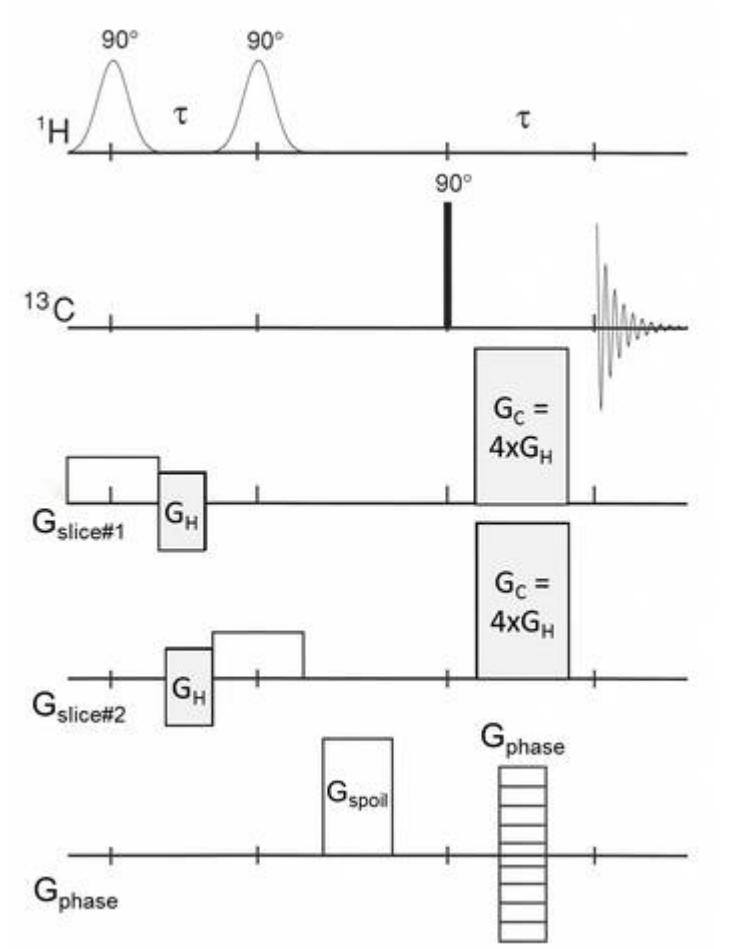

Figure 1. The heteronuclear STEAM sequence:
This {^{1}H-^{13}C} polarization transfer sequence is a variant of the STEAM localization. Two slice-selective ^{1}H pulses separated by the evolution interval τ, create hetero-nuclear longitudinal spin order I_zS_z, which is converted to ^{13}C coherences by a non-selective pulse. The third direction is encoded by phase gradients acting on ^{13}C. The homospoil gradient pulses during the τ interval (G_H,G_C) are weighted 1:4 according to the gyromagnetic ratios .

2.2 Pulse sequence

The proton localized {^{1}H-^{13}C} polarization transfer STEAM (PT-STEAM) sequence (Fig. 1) is a simplification of the refocused INEPT experiment: During the first *J*-evolution interval τ, antiphase proton magnetisation (e.g. I_xS_z for an AX system) evolves with *sin* (πJτ), yielding maximum PT at τ = (*n*+1/2)J – 1. A homospoil gradient pulse G_H replaces the 180° pulse for refocusing of proton chemical shifts. Thus, the second proton pulse creates gradient-spoiled longitudinal spin order (I_zS_z) superimposed by chemical shift evolution during τ. Polarisation transfer is achieved by the ^{13}C readout pulse creating antiphase carbon magnetisation (I_zS_x). The homospoil gradient pulse in the second τ-interval G_C is weighted 4:1 according to the gyromagnetic ratios of ^{1}H and ^{13}C. Like in the homonuclear STEAM sequence (Frahm et

al., 1987), the magnetisation created by PT is refocused with an inherent loss of 50%.

Localization is achieved by two slice-selective 90° sinc pulses of 2 ms duration at proton frequency, which prepare I_zS_z in the intersecting "rod" orthogonal to the coil plane. After a non-selective delayed 100 µs rectangular "read out" pulse at carbon frequency, 1D phase-encoding gradient was applied to localise signals in the direction perpendicular to the coil plane. When applying spatially selective 1H pulses, the newly created carbon S_x outside the proton localisation will be dephased by the large moment of G_C.

The spoiler gradients in τ were adjusted manually for maximum refocusing of the carbon signal transferred from proton magnetisation. In the *in vivo* experiments, the INEPT phase cycling scheme was applied for to eliminate spurious coherences that pass through the gradient selection although theoretically no phase-cycling is needed when applying gradient selection of coherence pathways (Hurd and John, 1991).

2.3 Experimental

To demonstrate signal enhancement and the effect of localization, a phantom containing a mixture of acetone, ethanol and chloroform was used, together with a sphere of 2.2 cm diameter containing chloroform which was fixed beneath the 13C coil.
Using gauss-shaped RF pulses of duration 1.0 ms, J-evolution intervals as short as $\tau = 4.2$ ms could be achieved to study the multiplet-dependent behavior ("J-filter") of the PT-STEAM signal. The experiment was run without localisation gradients to demonstrate the signal enhancement by PT, with and without 1H pulses. A value of 11.65 ms was chosen for τ, to be close to maximum polarisation transfer for both chloroform ($\tau = 11.35$ ms) and the acetone methyl groups ($\tau = 12.0$ ms).

2.4 Processing

Spectra were processed at the console using Bruker XWIN-NMR software. A mild Fermi-filter was applied on spatial phase-encoding and a Gaussian filter and zero-filling in time domain. Because the phase errors are no-longer linear in ^{13}C frequency spectra were displayed in magnitude mode and exported from XWIN-PLOT as postscript files.

3 Results

Due to the lack of 180° refocusing pulses, the difference of proton and carbon chemical shifts result in an arbitrary phase in the PT STEAM spectrum. They are thus displayed in magnitude mode (Fig. 2A). Note that the ^{13}C multiplets represent the net polarisation of the attached protons and are thus antisymmetric as they are not refocused. The carbon singlet resonance of acetone and the central peak of the methylene triplet are suppressed.

3.1 Enhancement and J-filter

The magnitude spectrum of a pulse-and-acquire experiment in Fig. 2A shows the multiplet structure:

the CO singlet of acetone at 205 ppm chemical shift and the methyl quartet at δ = 30 ppm (*J*~125 ms). This is partly overlapping with the methylene triplet of ethanol (δ = 57 ppm, *J*~125 ms), but well resolved from the chloroform doublet at 78 ppm (J~220 ms).

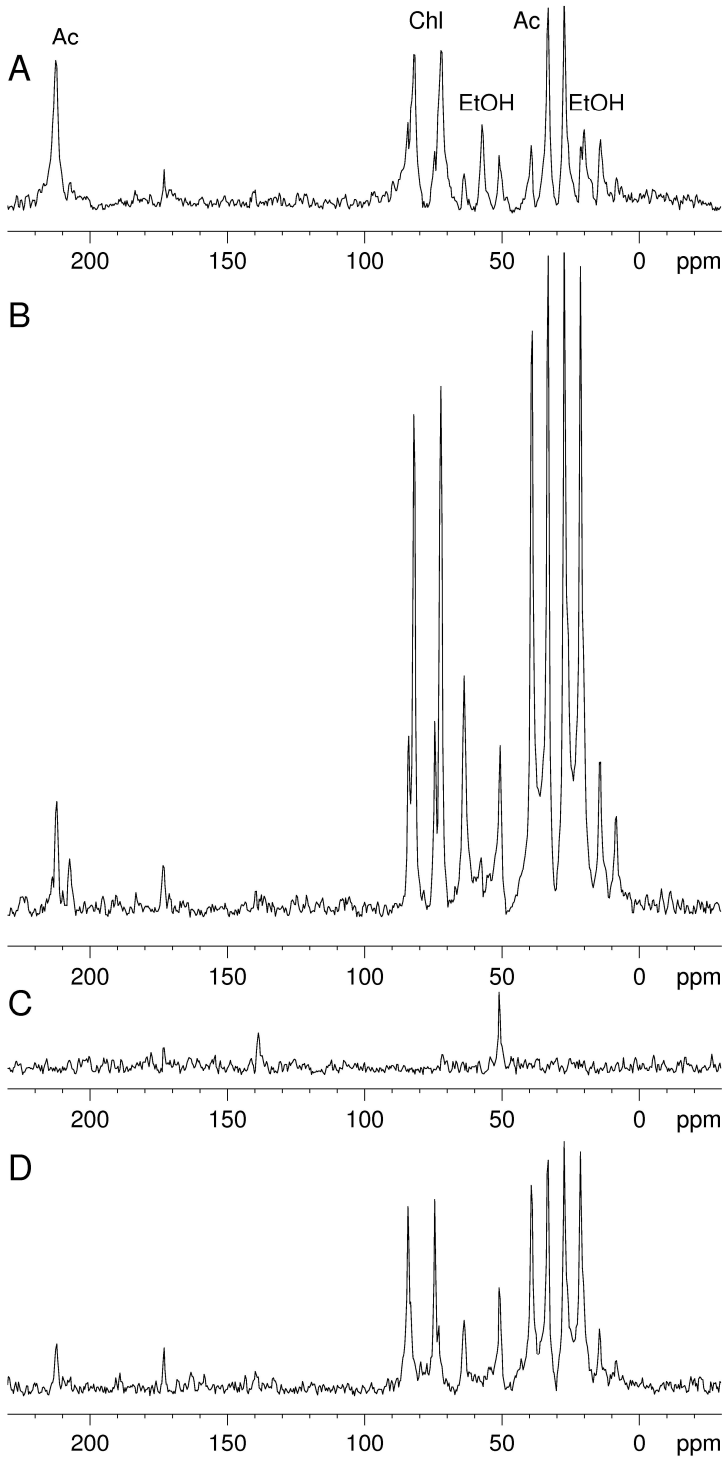

Figure 2.

Signal gain by {^{1}H-^{13}C} STEAM: Ac = acetone, Chl = choroform, EtOH = ethanol. Magnitude displayed.

A: Non-localised pulse-and-acquire experiment using a ^{13}C pulse of 100 µs. A slight shift separates the Chl signals originating from two separate phantoms.

B: Spectrum obtained with the non-localised PT-STEAM sequence and the same ^{13}C pulse as in A.

C: Control experiment without proton RF pulses.

D: Localised spectrum from a $(2.88)^2$×0.5 ml voxel excluding the external Chl signals.

To demonstrate the signal enhancement by PT, the experiment was run without localisation gradients (Fig. 2B). $\tau = 11.65$ ms was chosen to be close to maximum polarisation transfer for both chloroform ($\tau = 11.35$ ms) and acetone ($\tau = 12.0$ ms). This yielded a 3.3-fold signal gain for the chloroform doublet, i.e. about a factor of four (for an AX system) diminished by refocusing and relaxation losses. The acetone resonances showed a similar enhancement. The ethanol resonances were enhanced less, due to the homonuclear J -coupling of the protons.

The control experiment with zero amplitude of the proton pulses in Fig. 2C proved that the spectrum in Fig. 2B is almost exclusively due to polarisation transfer. This signal gain allows additional localisation with satisfactory signal-to-noise ratio. The localised spectrum in Fig. 2D shows slightly bigger amplitudes than the non-localised spectrum in Fig. 2A, though it is acquired with full localisation corresponding to $0.5*2.88^2 = 4.1$ ml. This may be explained 1D CSI introducing an arbitrary factor via the discrete Fourier transform.

J-evolution of the multiplets takes place during the two τ intervals, creating a J-filter. By choosing an appropriate τ resonances of interest can be enhance while reducing unwanted resonances. In Fig. 3A the chloroform doublet is suppressed at $\tau = 4.5$ ms; Fig. 3B the ethanol and acetone resonances ($\tau = 8.0$ ms).

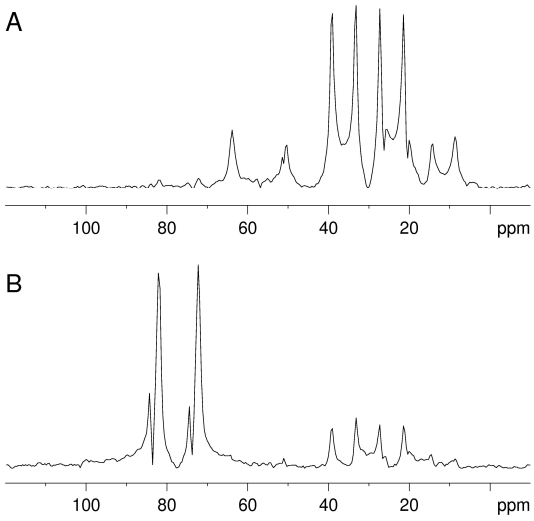

Fig 3: Spectral editing by J –filter: A: Suppression of the chloroform signal at $\tau = 4.6$ ms B: Suppression of the ethanol and acetone at $\tau = 8.0$ ms

3.2 Spatial and spectral resolution

Using slice selective proton excitation and 16 phase encoding steps with 8 cm FOV orthogonal to the coil plane, spatial resolution of the signals orthogonal to the coil plane was accomplished. This corresponds to 3D localisation of each ^{13}C spectrum with reduced chemical shift displacements. Figure 4A shows the depth profile of the signal from a $(28.8 \text{ mm})^2$ cross-section of the homogeneous phantom ($\tau = 11.65$ ms). The chloroform signal from the external phantom is clearly resolved, demonstrating the

simultaneous acquisition of a reference signal of known concentration.

For initial application in vivo, 32 phase encoding steps were acquired within 12 minutes (TR = 1.4 s) from a $(45\ mm)^2$ cross-section into human calf muscle. The *J*-evolution interval $\tau = 9$ ms has been chose with regard to the CH coupling of the fat resonances. Thus depth resolving of lipid signals was achieved. The 12 adjacent spectra shown in Fig. 4B correspond to a depth of 3 cm.

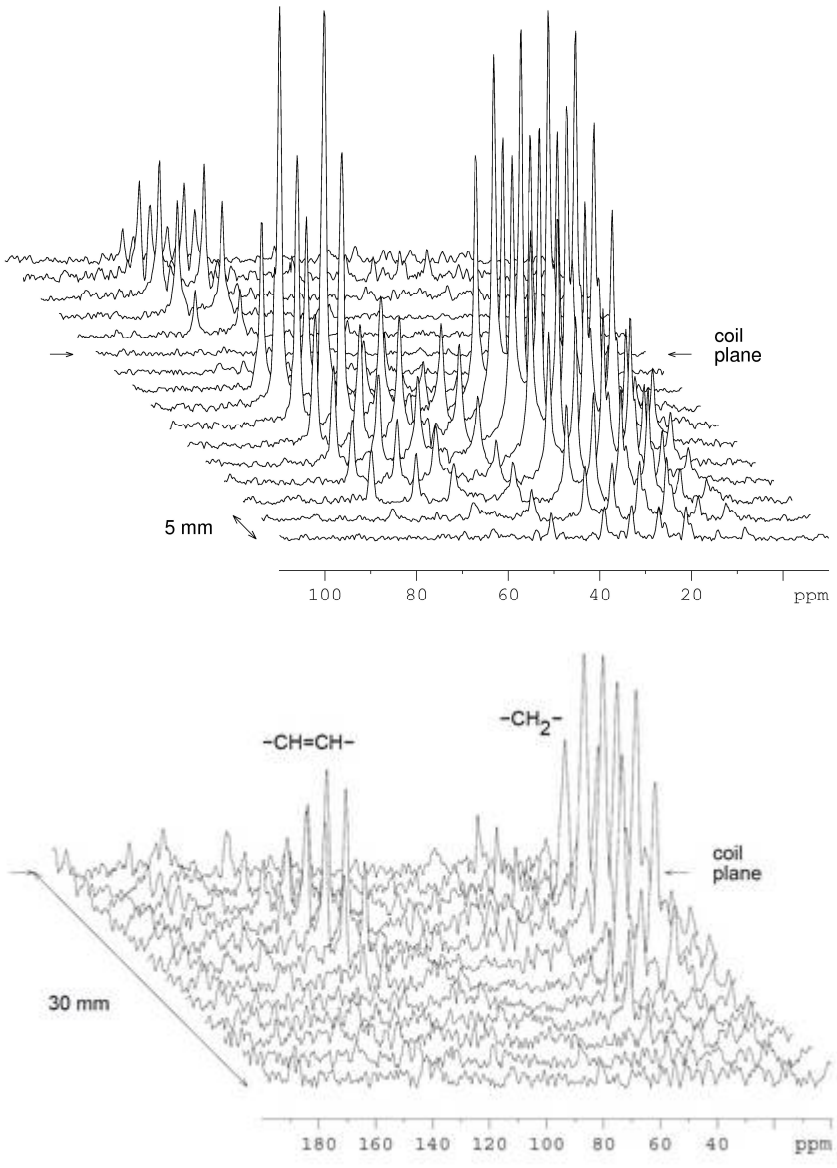

Figure 4. Spatial-spectral resolution of PT-STEAM:
A: Depth profile of excitation and reception demonstrated on the homogeneous phantom containing ethanol. $(28.8\ mm)^2 \times 5$ mm SI resolution. The Chl doublet in the back rows originates from the external reference.
B: 1D SI of human calf muscle. $(45\ mm)^2 \times 2.5$ mm SI resolution. 12 minutes measuring time. $\tau = 9.0$ ms was adjusted for detection of lipid CH_2 resonances.

4. Discussion

The key feature of the PT-STEAM sequence is the use of homospoil gradients for coherence pathway selection. It is based on the INEPT experiment, because in DEPT polarisation transfer is mediated by multiple quantum coherences (Doddrell et al., 1982), which differ in their sensitivity to gradients. The sequence was a considerably simplified by removing the refocusing pulses which benefits both implementation on whole body MR systems and thus *in vivo* applications on humans (Beckmann and Müller, 1991). Because of gradient pathway selection no phase cycling is needed and subtraction errors due to movement or system instability are avoided. With sufficient dephasing of the proton magnetisation, the polarisation transfer becomes independent of the chemical shift evolution during τ as shown for the STEAM experiment (Frahm et al., 1989). Therefore, the 180° pulses inherent to INEPT for chemical shift refocusing may be removed, resulting in fewer gradient switches and a reduction to only three RF pulses.

Separation of the proton and carbon pulses makes the sequence suitable for double tuned volume coils. Since polarisation transfer is mediated via longitudinal spin order the central mixing delay does not contribute to T_2 losses. The resulting sequence can be regarded as a kind of heteronuclear STEAM experiment, with the inherent 50% signal loss.

Note that the ^{13}C multiplets represent the net polarisation of the attached protons and therefore are antisymmetric. The carbon singlet resonance of acetone and the central peak of the methylene triplet are suppressed. Polarisation transfer and the *J*-evolution of antiphase magnetisation depend on the spin system. If $\tau = (n+1/2)J^{-1}$ (like in this implementation), an AX doublet is refocused, an AX_2 system has evolved back to antiphase, and the phases of an AX_3 system appear as +,–,–,+. A definite drawback is the phase error introduced by the evolution of proton and carbon chemical shift during τ, which makes magnitude display of the spectra necessary. Overlap of multiplets may give rise to unwanted cancellation. A possible countermeasure is to extend the sequence to form a symmetric double inverse HSQC experiment (Watanabe et al., 1996). The three proton pulses offer 3D gradient localisation and a stronger enhancement. As the amplitude of RF irradiation is limited on clinical MR systems, the duration of 180° pulses cannot be reduced under a certain limit and therefore increase T_2 losses due to prolonged *J*-evolution intervals.

The range of *J* values of in vivo detectable metabolites is limited from 120 Hz for methyl groups to more than 160 Hz for aromatic systems. This corresponds to optimal values of τ as short as 3 ms, which may be achieved with faster and stronger gradient systems. A reduction in τ to 3.5 ms (compromising for the simultaneous detection of all metabolites) would be possible by the use of shorter pulses, e.g. of gaussian shape. Without a time consuming selective pulse, the second τ-interval provides more time to apply the fourfold gradient pulses for refocusing of ^{13}C magnetisation. Although the selectivity of the *J*-filter may be improved by the choice of longer τ, the limited range of *J* values of *in vivo* relevant metabolites is likely to results to a concomitant suppression of the whole spectrum.

By applying 1D phase encoding gradients on the ^{13}C magnetisation, we have shown that the PT-STEAM sequence is capable of achieving a 3D localisation of carbon spectra. Thus, resonances originating from different locations may be separated. This allows for an acquisition of both the *in vivo* spectrum and a reference signal from an external phantom in one experiment, even in the presence of dominating contributions from subcutaneous tissue. By means of ^{1}H localisation of a rectangular column,

the influence of lateral sensitivity gradients of the surface coil are reduced facilitating quantification.

As the measuring time *in vivo* was shown to be less than 15 minutes, even on an outdated MR system, the sequence may be useful for clinical examinations. The study of the brain or underlying organs in the abdomen will be possible with the use of an appropriate proton/carbon resonator. Fat suppression can be achieved by discarding the spectra from subcutaneous locations and/or an appropriate *J*-filter.

The unrestricted mixing delay allows for inverting the 1H polarisation, as refocused multiplets are a pre-requisite for Waltz decoupling.

This note presents the basic concept of a hetero-nuclear STEAM sequence. Further refinements were not explored. The mixing delay is less constrained and can thus accommodate additional inversion pulses. Finally, by encoding of the evolution of ^{13}C magnetisation, overlapping ^{1}H resonances may be separated by the pertinent ^{13}C chemical shifts in a heteronuclear 2D correlation spectrum as an alternative approach to separating signals by their spatial origin.

5. Acknowledgements

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