Structure Elucidation of Heparin Adulteration

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Laboratorium
für Auftragsanalytik GmbH

A NMR Study by Dr. B. Diehl and Dr. G. Randel, April 2008
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Approximately 30 Heparin samples were tested for adulteration

Summary: Oversulfated Chondroitin Sulfate A/C (OSCO) ex porcine sources was successfully identified by high temperature ^{1}H NMR (HTHNMR) at 353 K (see slight 1) and characterised by 1- and 2-dimensional methods using the whole adulterated material. The adulteration material was isolated by precipitation from aqueous contaminated Heparin samples by successive addition of methanol (see slight 7).

Comparison of the spectral data with reference material of possible chemical structures was performed according to the following list.

Hyaluronic Acid (see slight 8)
Chondroitin Sulfate B (Dermatan Sulfate) (see slight 9)
Chondroitin Sulfate A/C ex porcine mucosa (see slight 10)

The chemical shifts of all N-Acetyl-groups of these substances appear at δ = 2.0 very close to the Heparin related signal itself. The downfield shift of the adulteration acetyl group at δ = 2.15 ppm must be caused by additional anisotropic effects. An O-acetylation was excluded by HMBC and HMQC spectra. MS data of the hydrolysed precipitate indicate a high sulfatation of the polymer. Therefore a synthetic approach was performed preparing oversulfated Dermantan Sulfate and Chondroitin Sulfate. Synthesis was successfully performed at INNOVENT e.V. technology development, Jena.

Sulfated Dermatan Sufhate + 1 SO_3 (see slight 12)
Oversulfated Dermatan Sulfate + 3 SO_3 (see slight 13)
Oversulfated Chondroitin Sulfate A/C ex porcine mucosa (see slight 14)

The chemical shifts of the two oversulfated species fit the N-acetyl signal of the adulteration. The OSCS fit the spectrum of the adulteration in all chemical shifts **(see slight 15)**. Even fine structures can be obtained to be identical.
The results confirm the conclusion of the independent study published in Nature Biotechnology Advanced Online Publication, 23. April 2008.

Comparison of 300 MHz HTHNMR (top) and 500 MHz at RT

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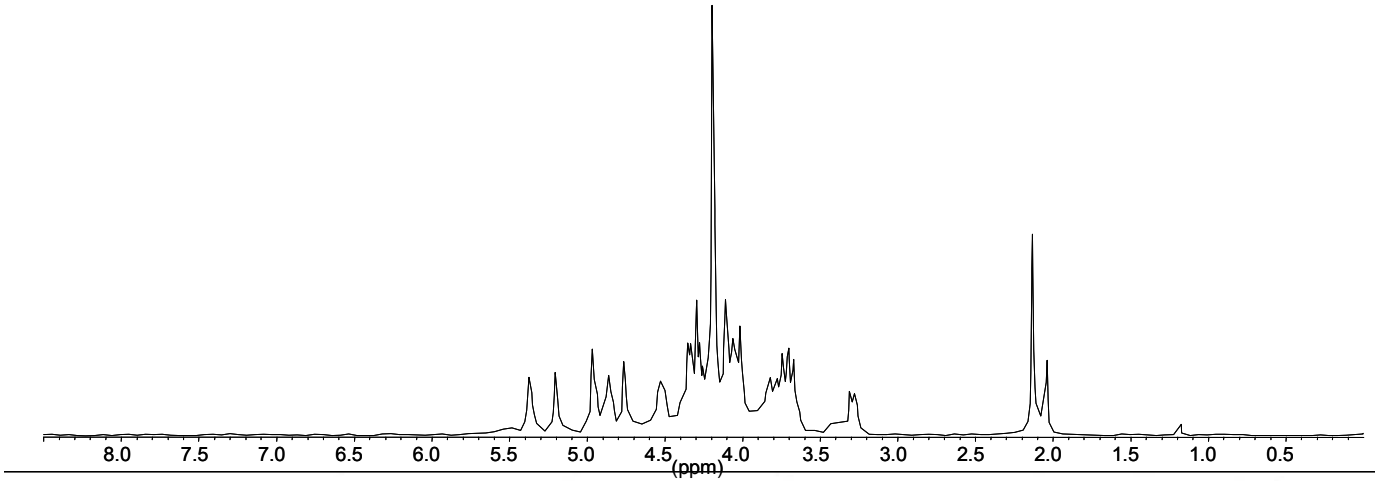

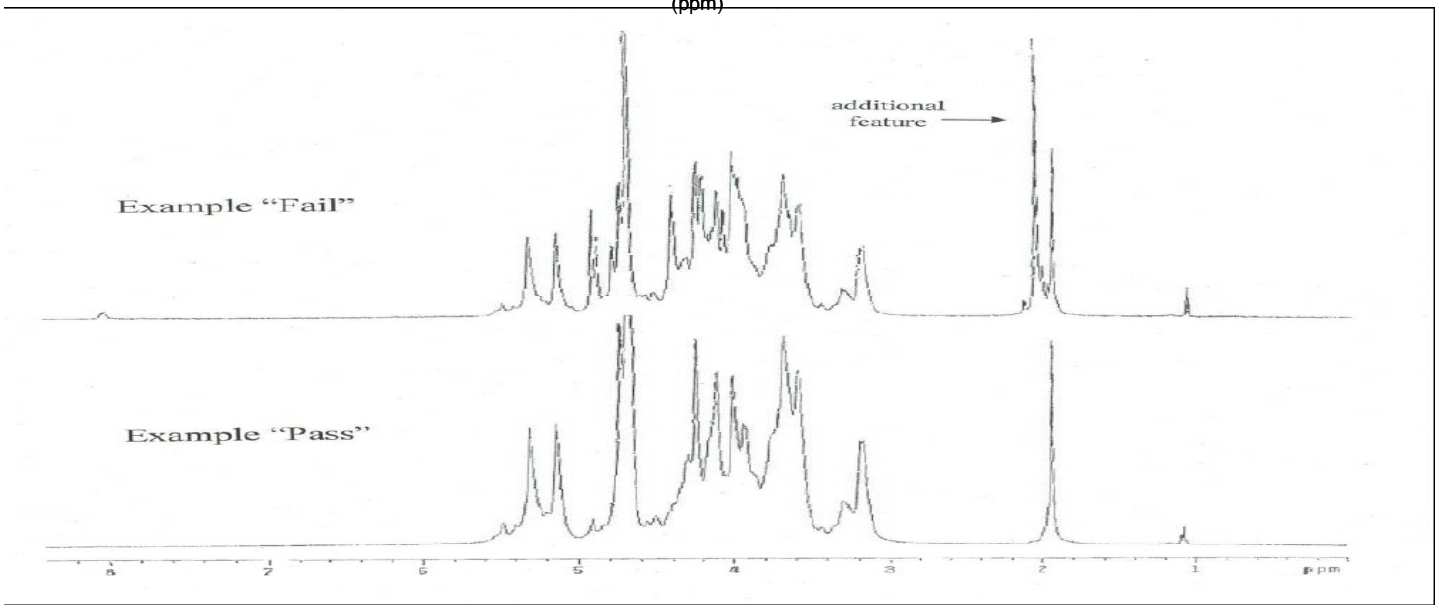

HTHNMR of pure Heparin at 353 K

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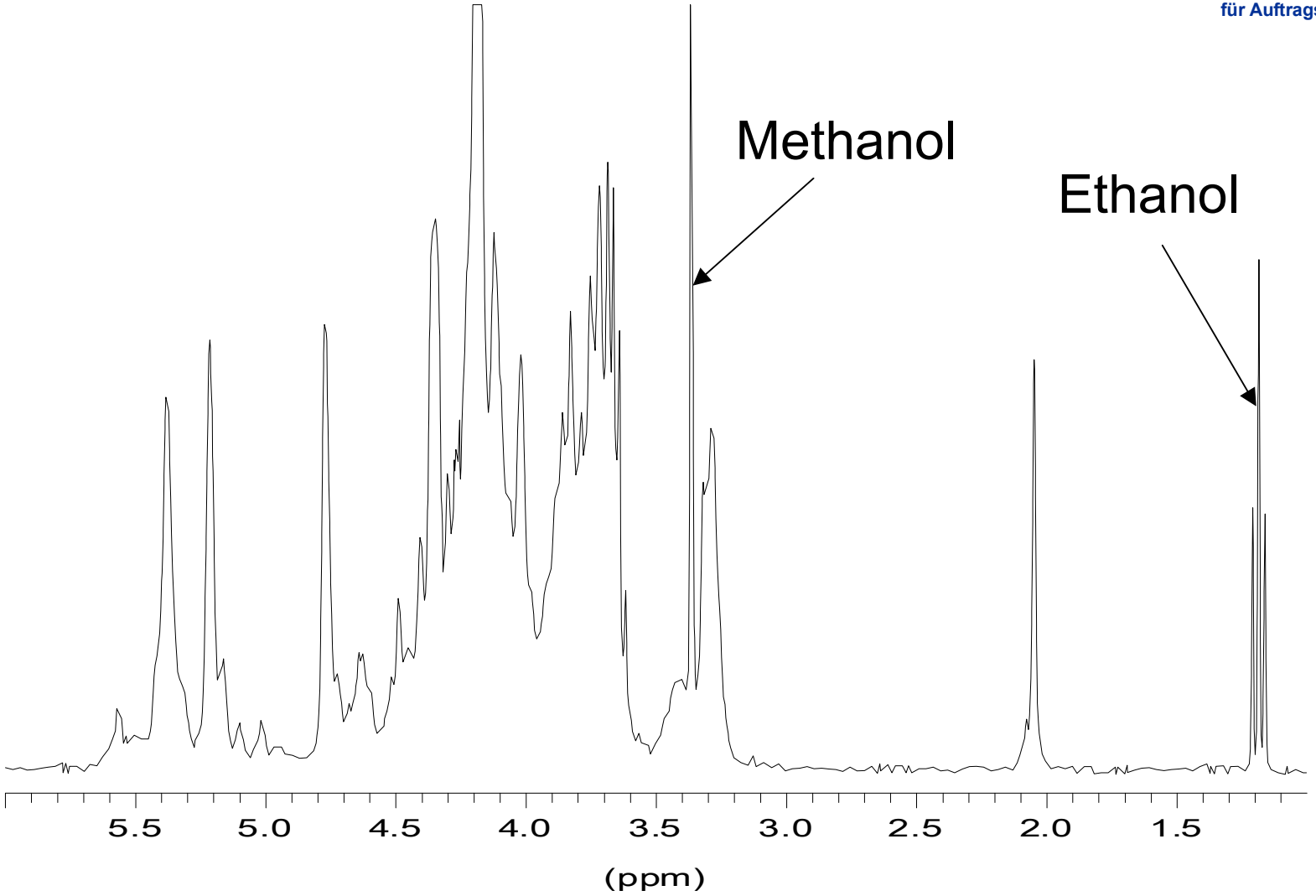

HTHNMR of pure Heparin with Dermatan Sulfate at 353 K

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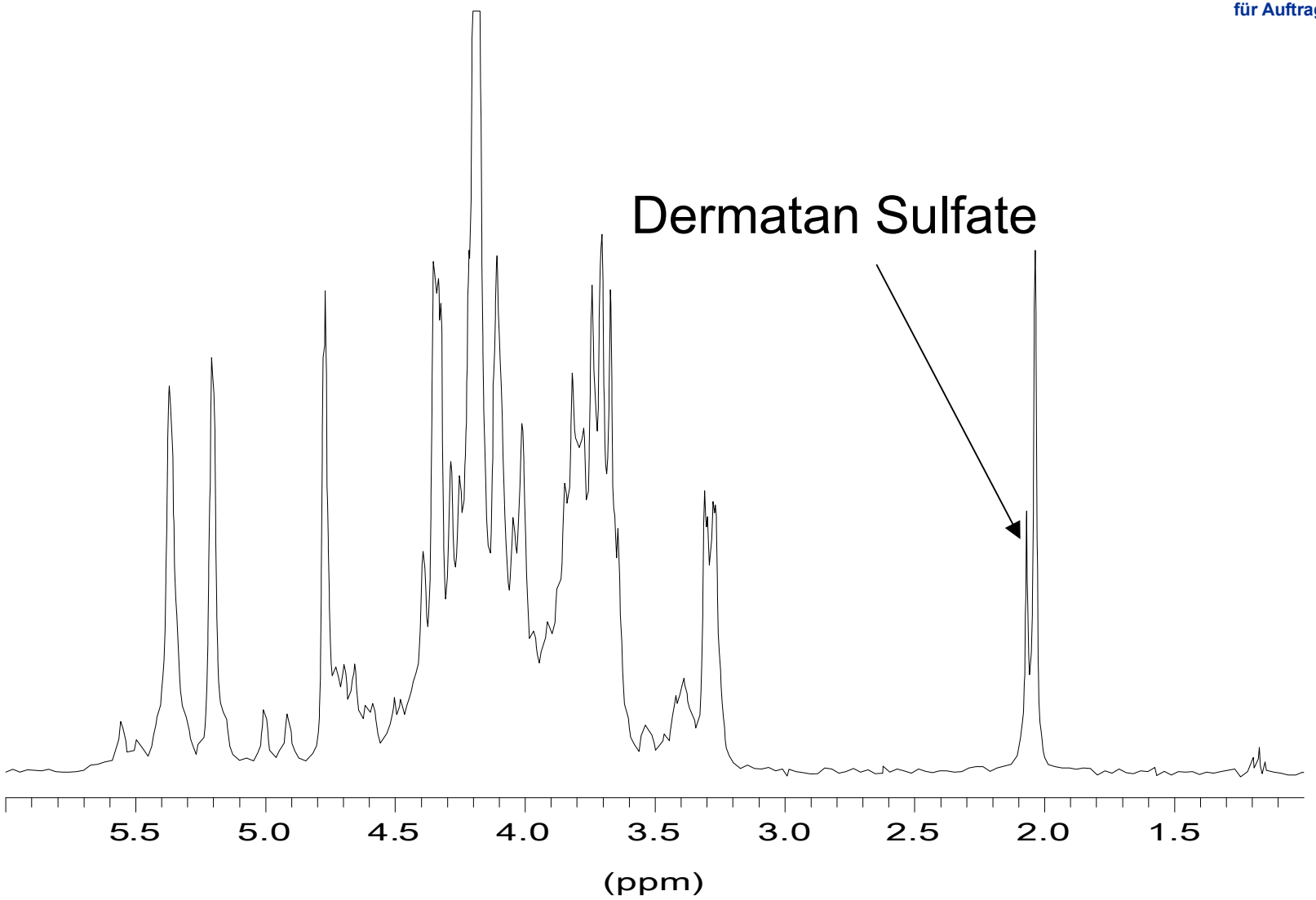

HTHNMR of adulterated Heparin at 353 K

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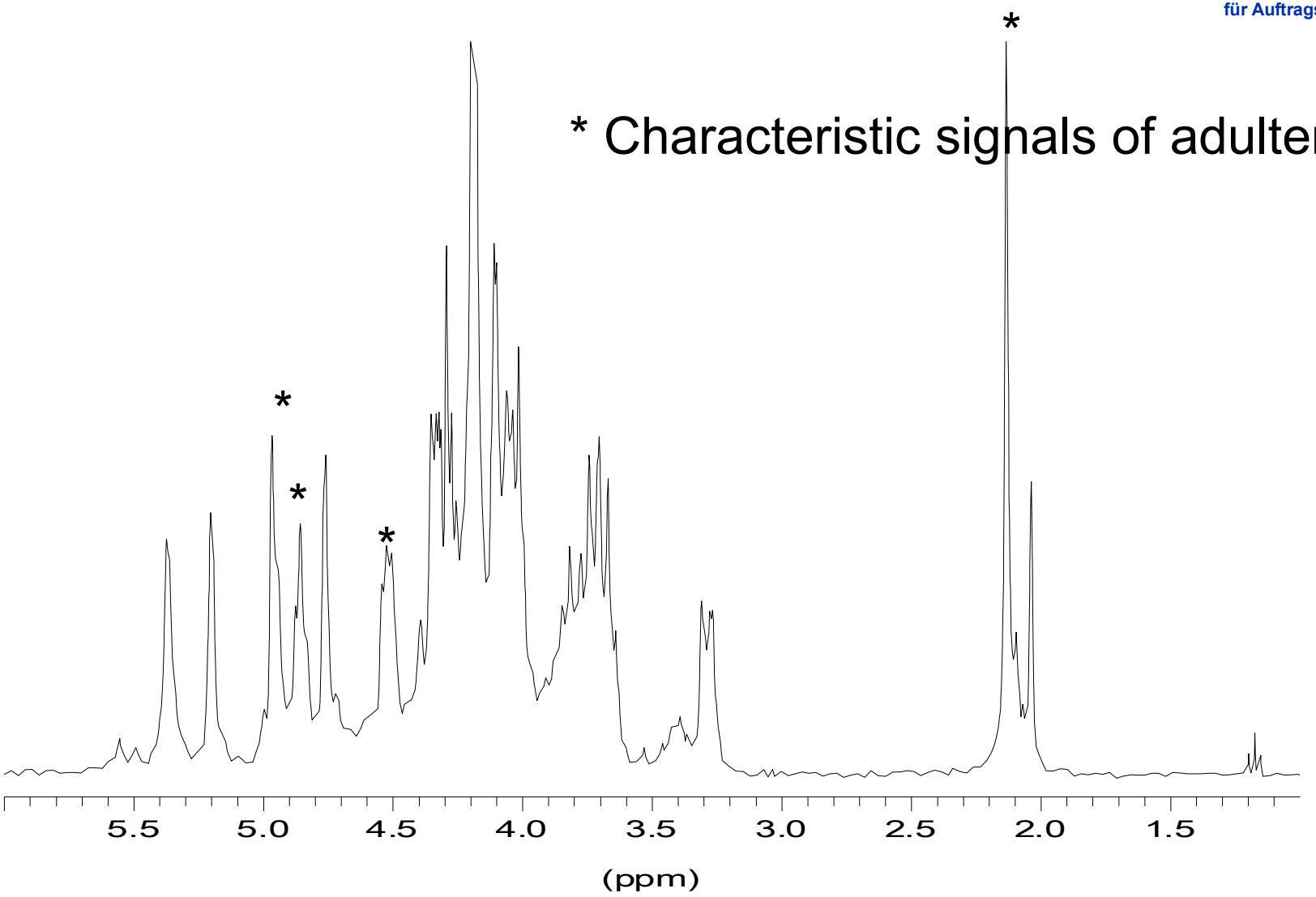
* Characteristic signals of adulteration



The amount of adulteration quantified by standard addition

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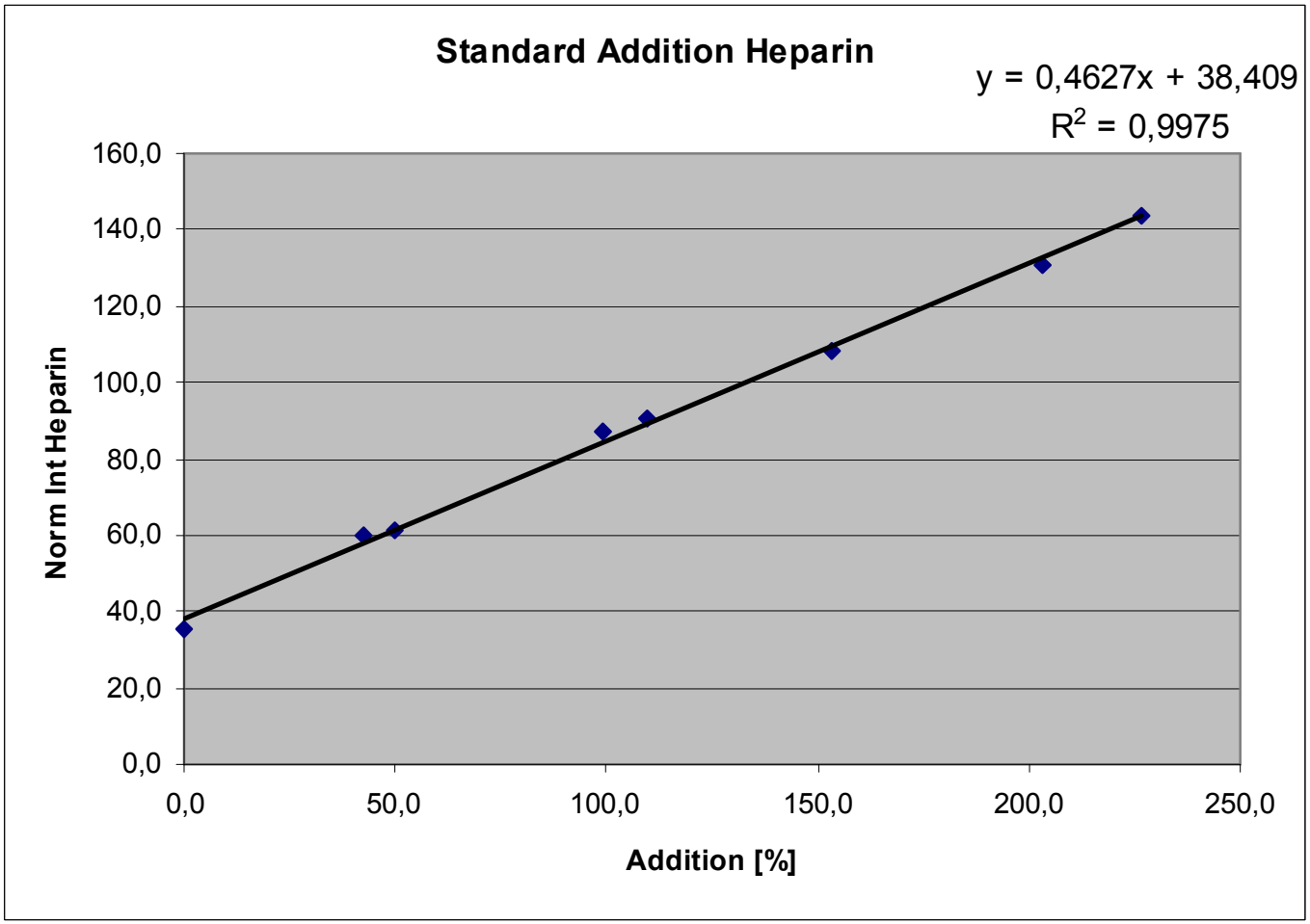

HTHNMR of isolated adulteration at 353 K

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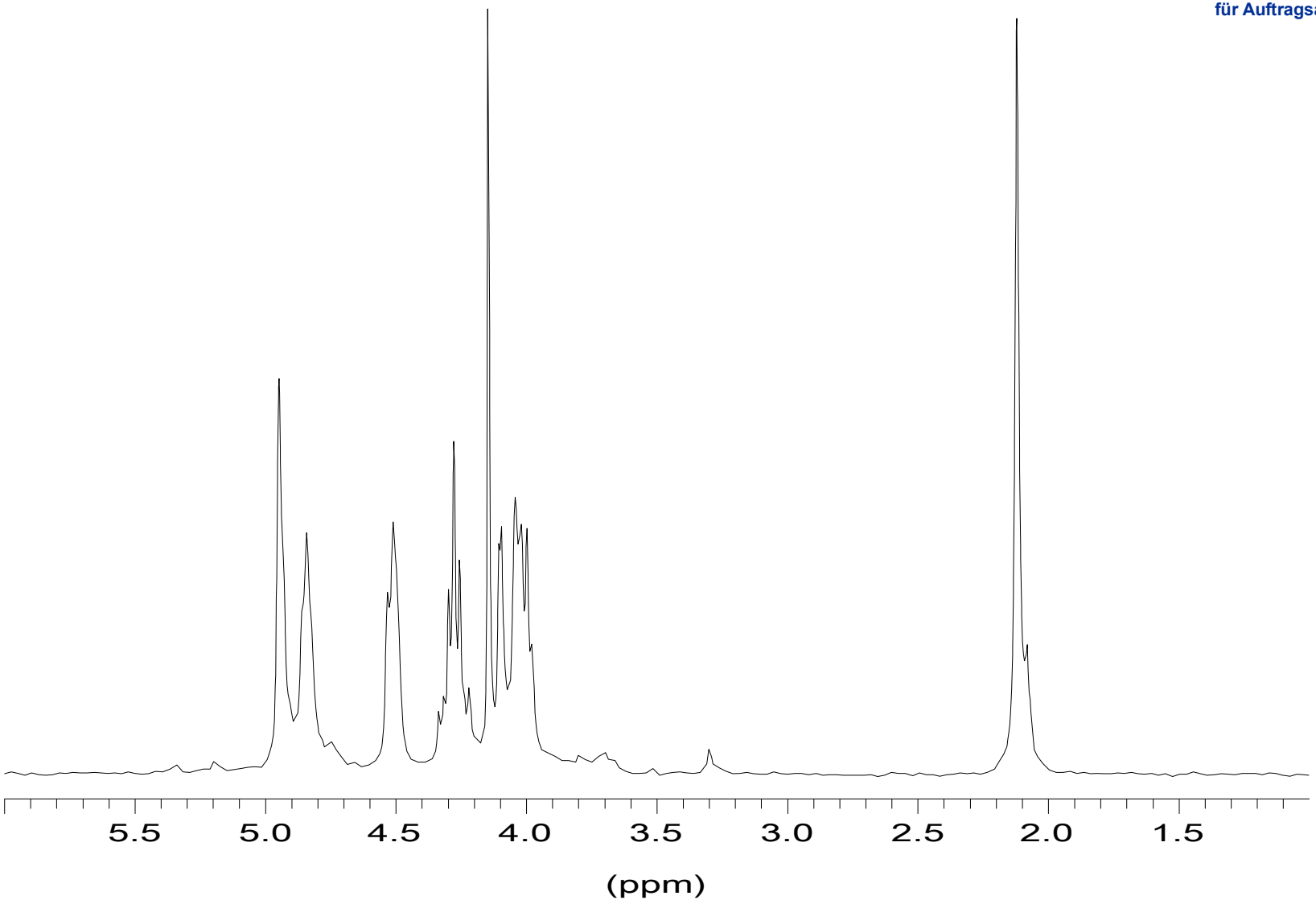

^{1}H-NMR of Hyaluronic Acid at RT

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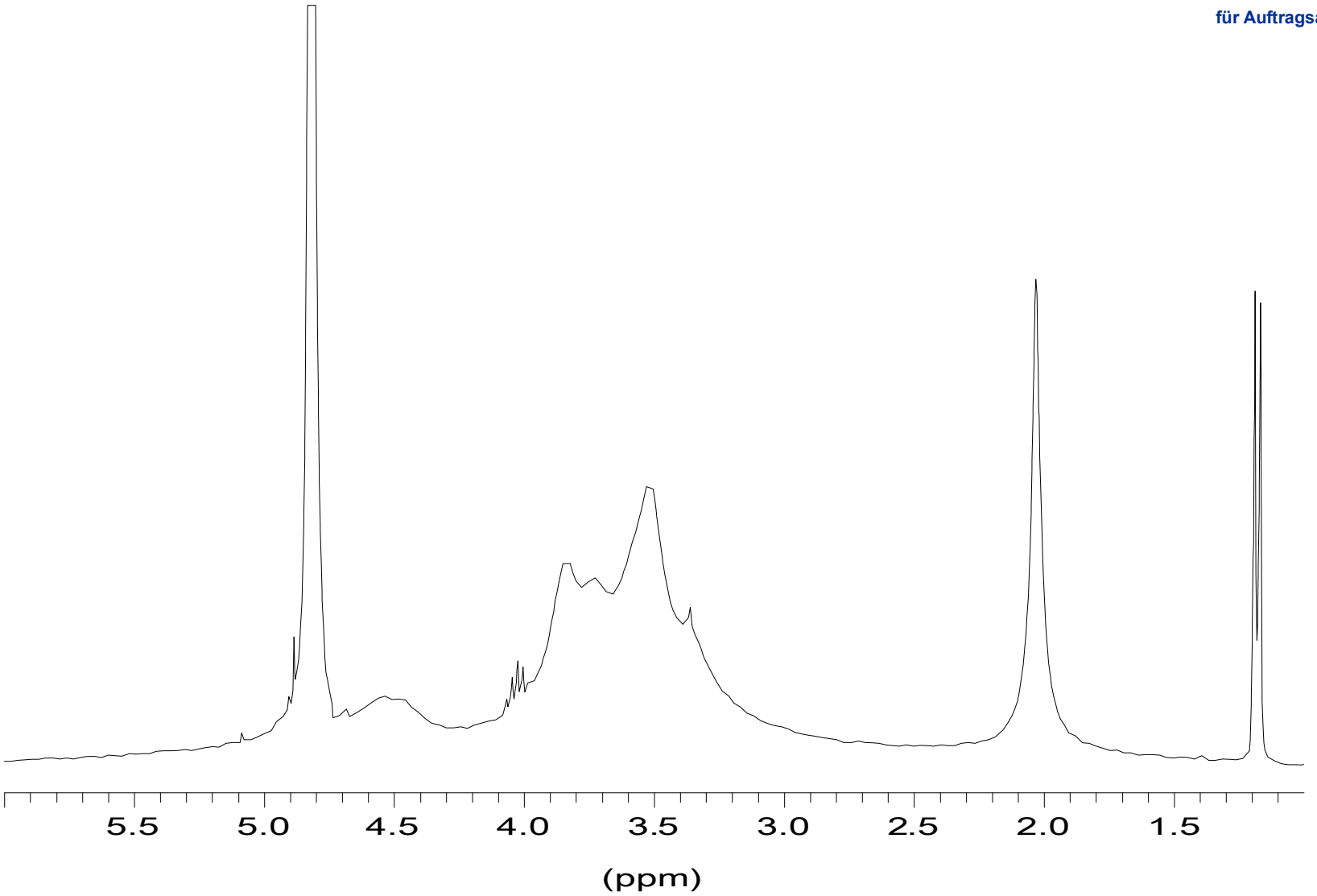

^{1}H-NMR of Dermatan Sulfate at RT

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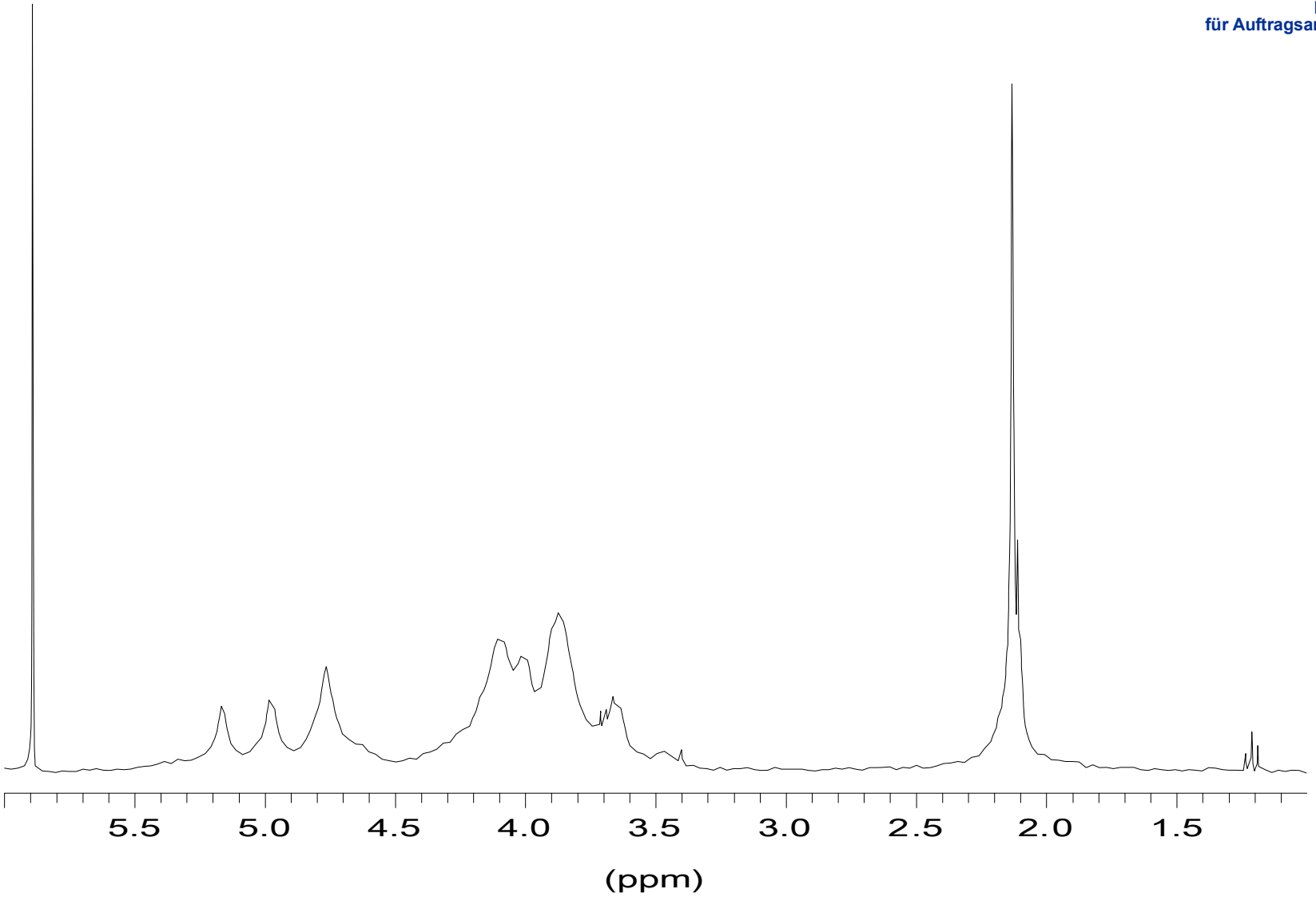

HTHNMR of Chondroitin Sulfate A/C (CS) at 353 K

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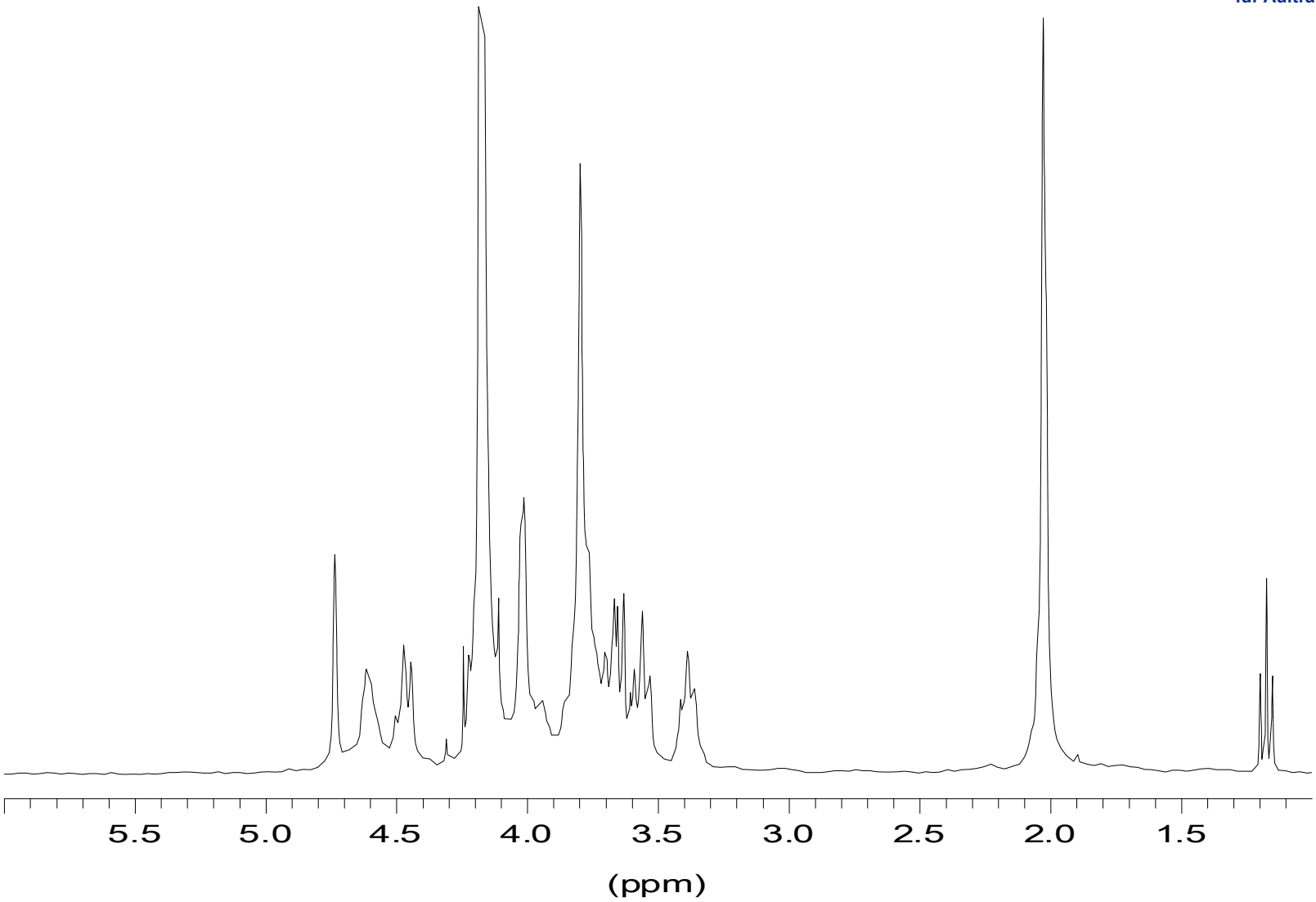

Comparison of HTHNMR spectra of CS (top) and isolated adulteration (bottom) at 353 K

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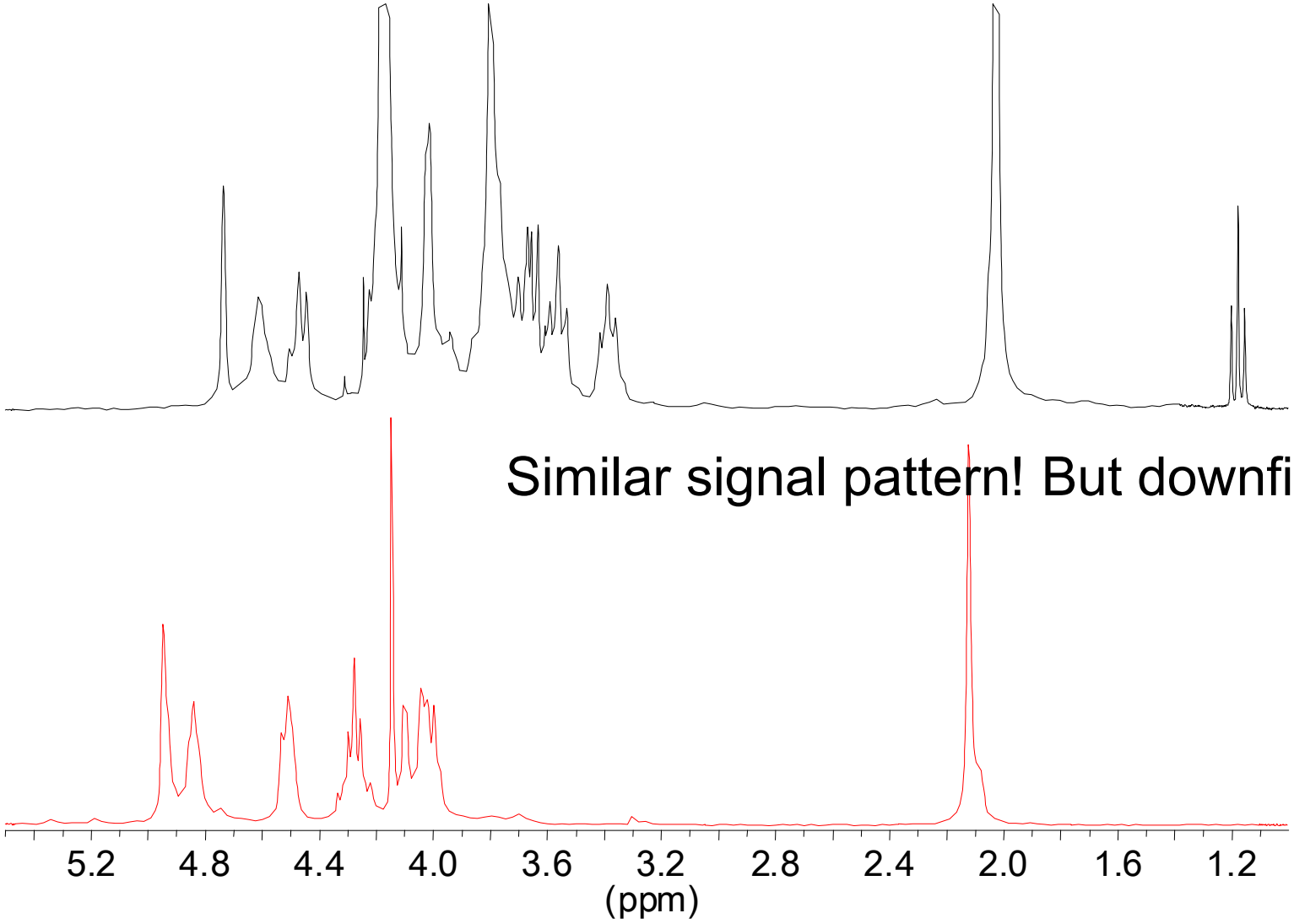

Similar signal pattern! But downfield shifts

^{1}H-NMR of Sulfated Dermatan Sulfate at RT

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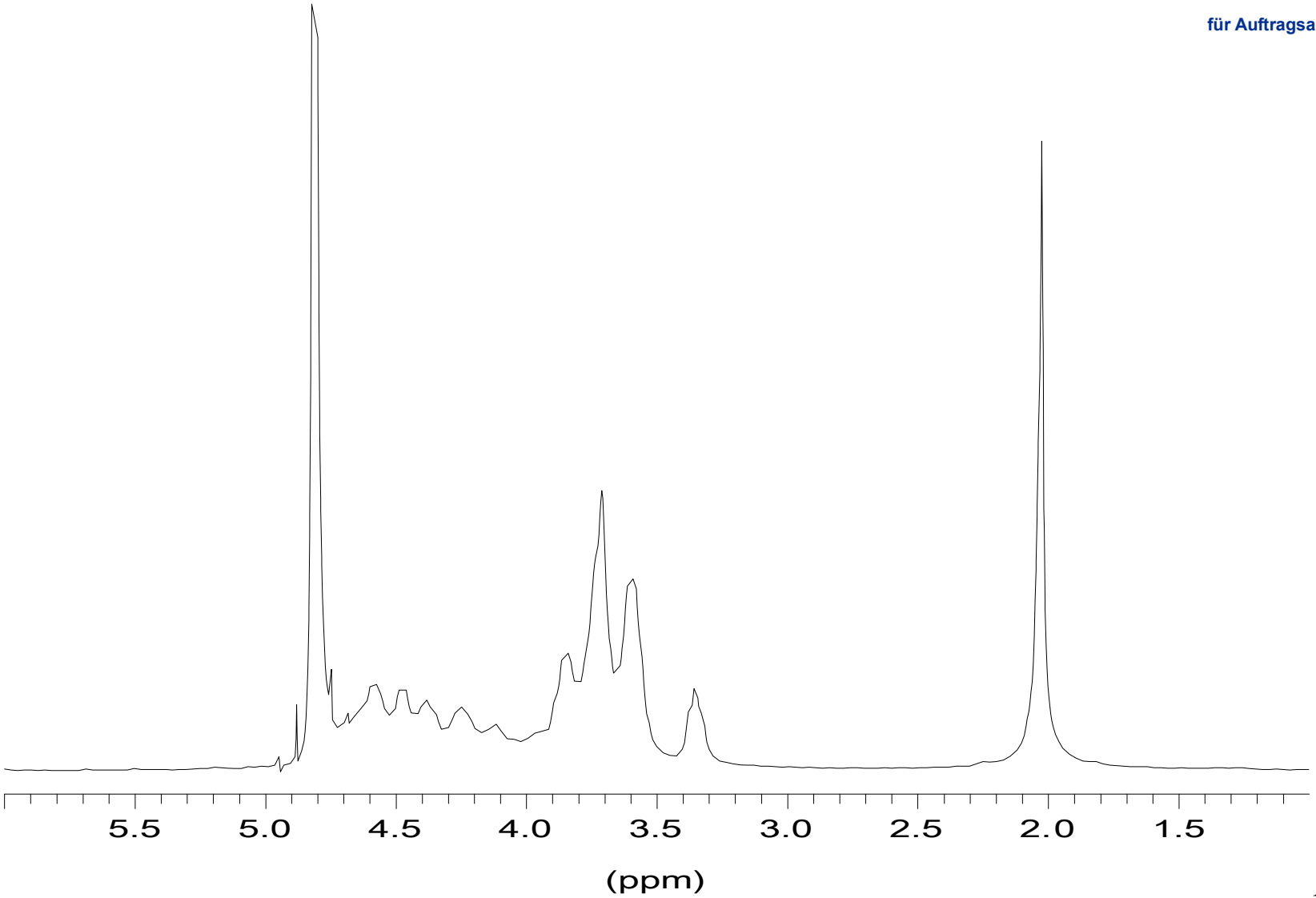

^{1}H-NMR of Oversulfated Dermatan Sulfate at RT

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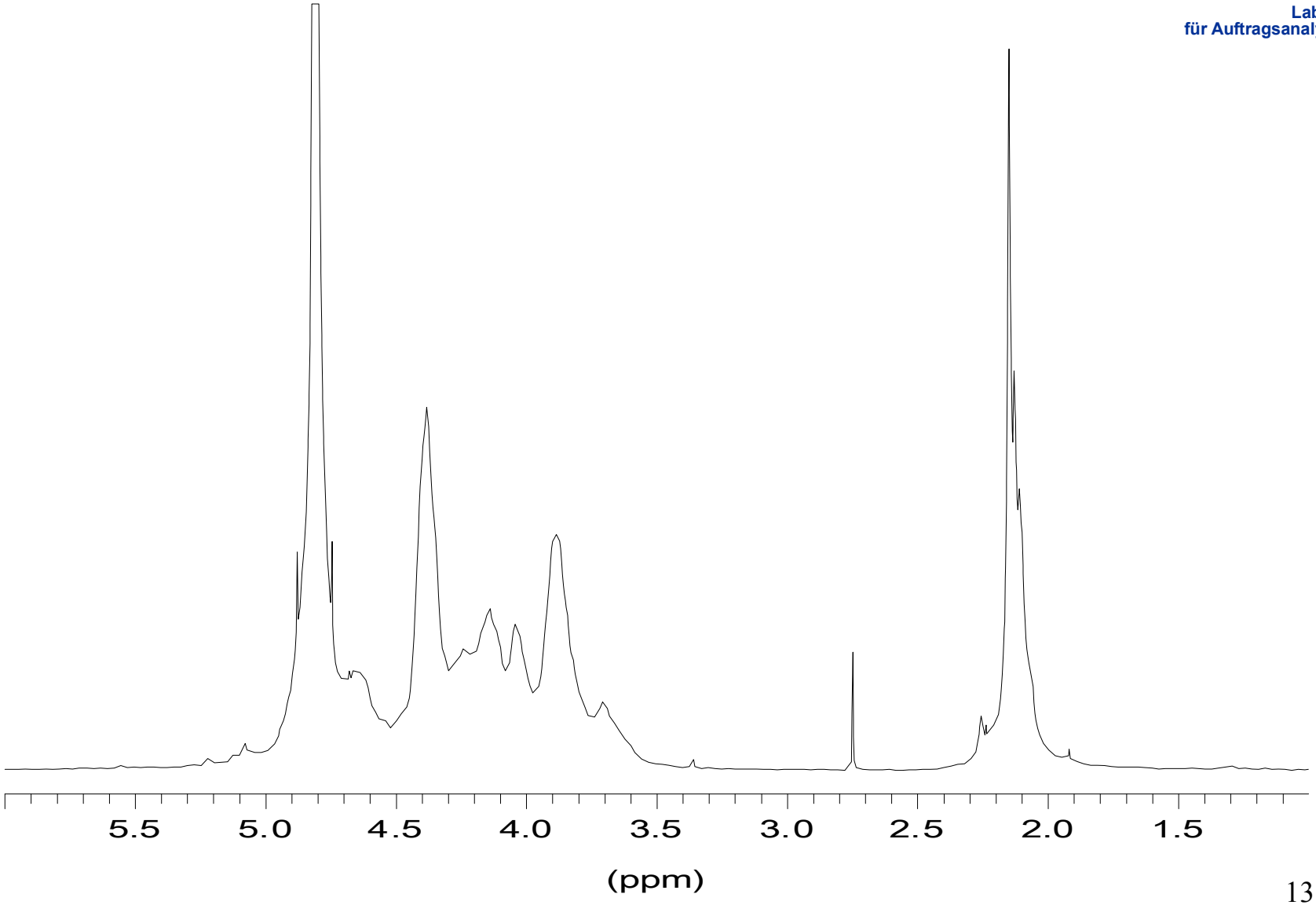

^{1}H-NMR of Oversulfated CS at RT

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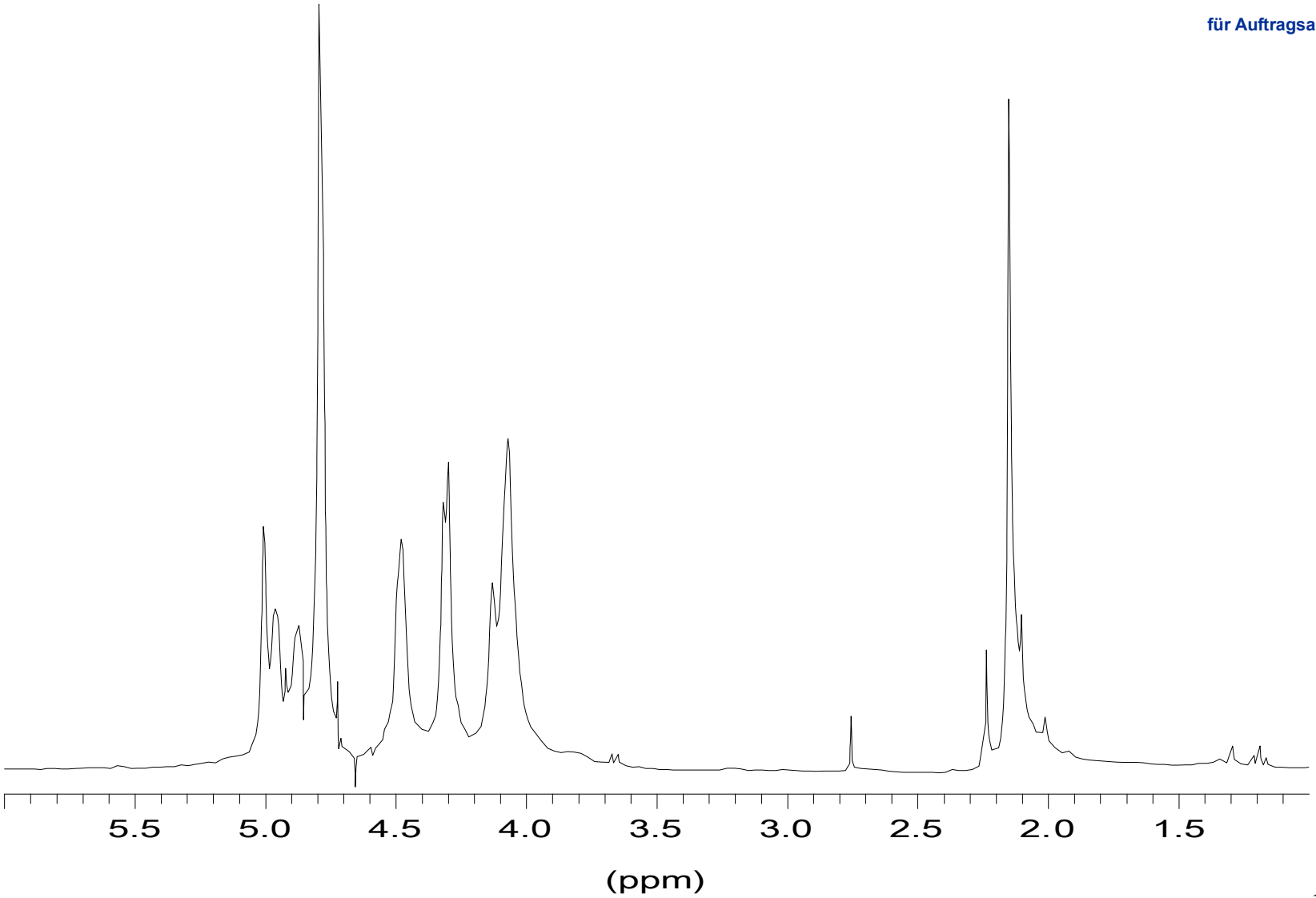

Comparison of ^{1}H-NMR spectra of Oversulfated CS (top) and isolated adulteration (bottom) at RT

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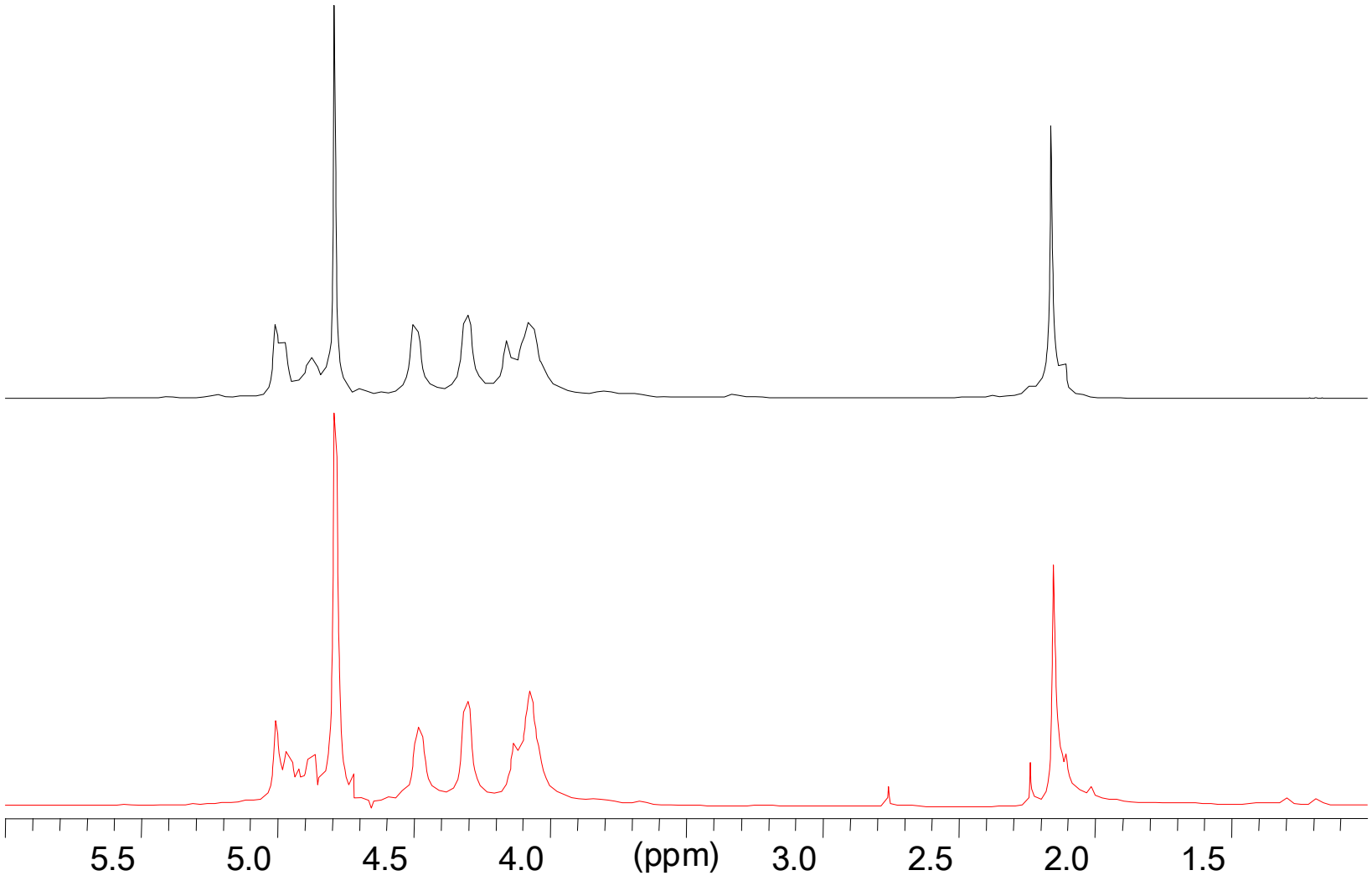

HTHNMR of Heparin spiked with CS at 353 K

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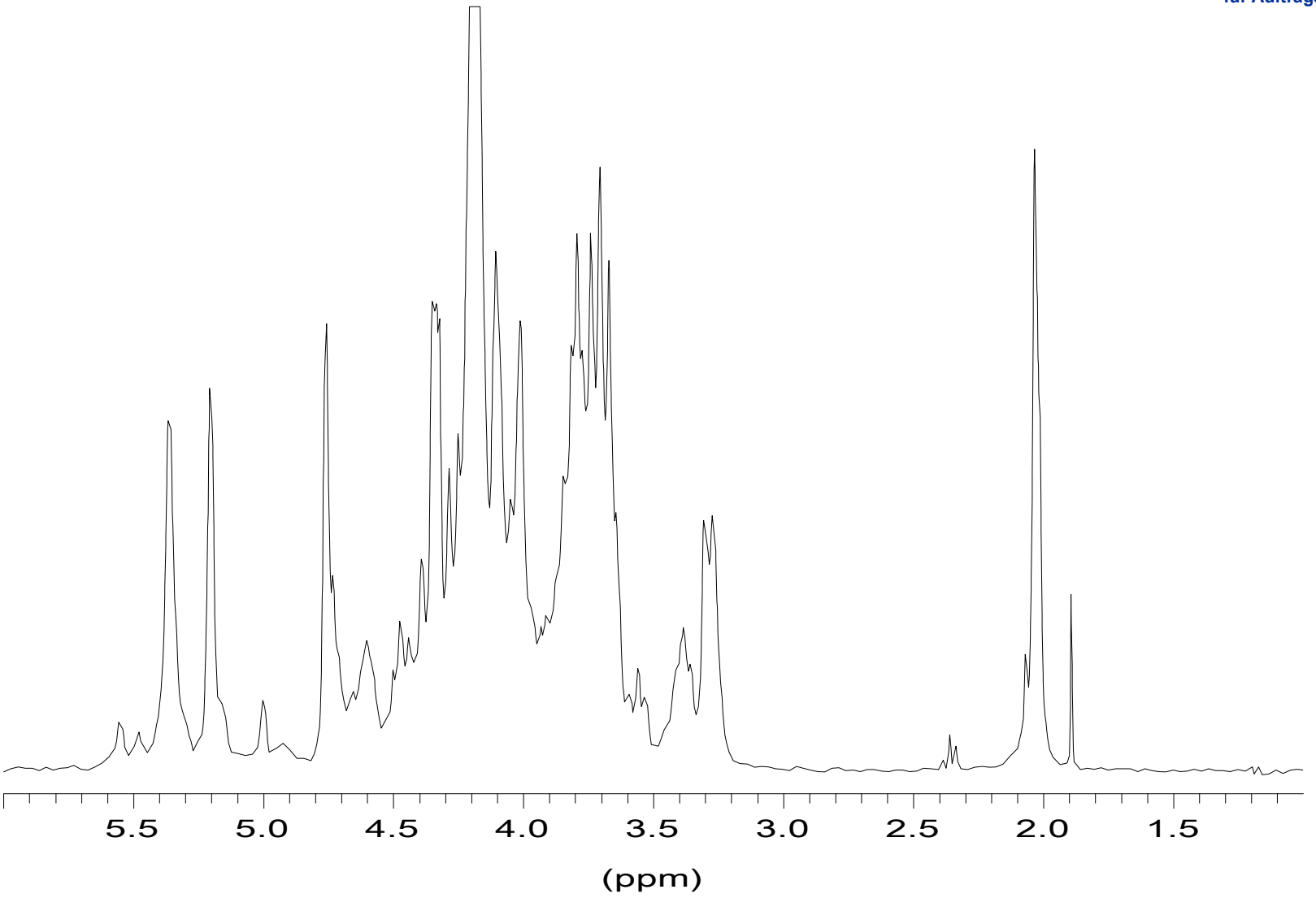

HTHNMR of Heparin spiked with CS at 353 K, detail

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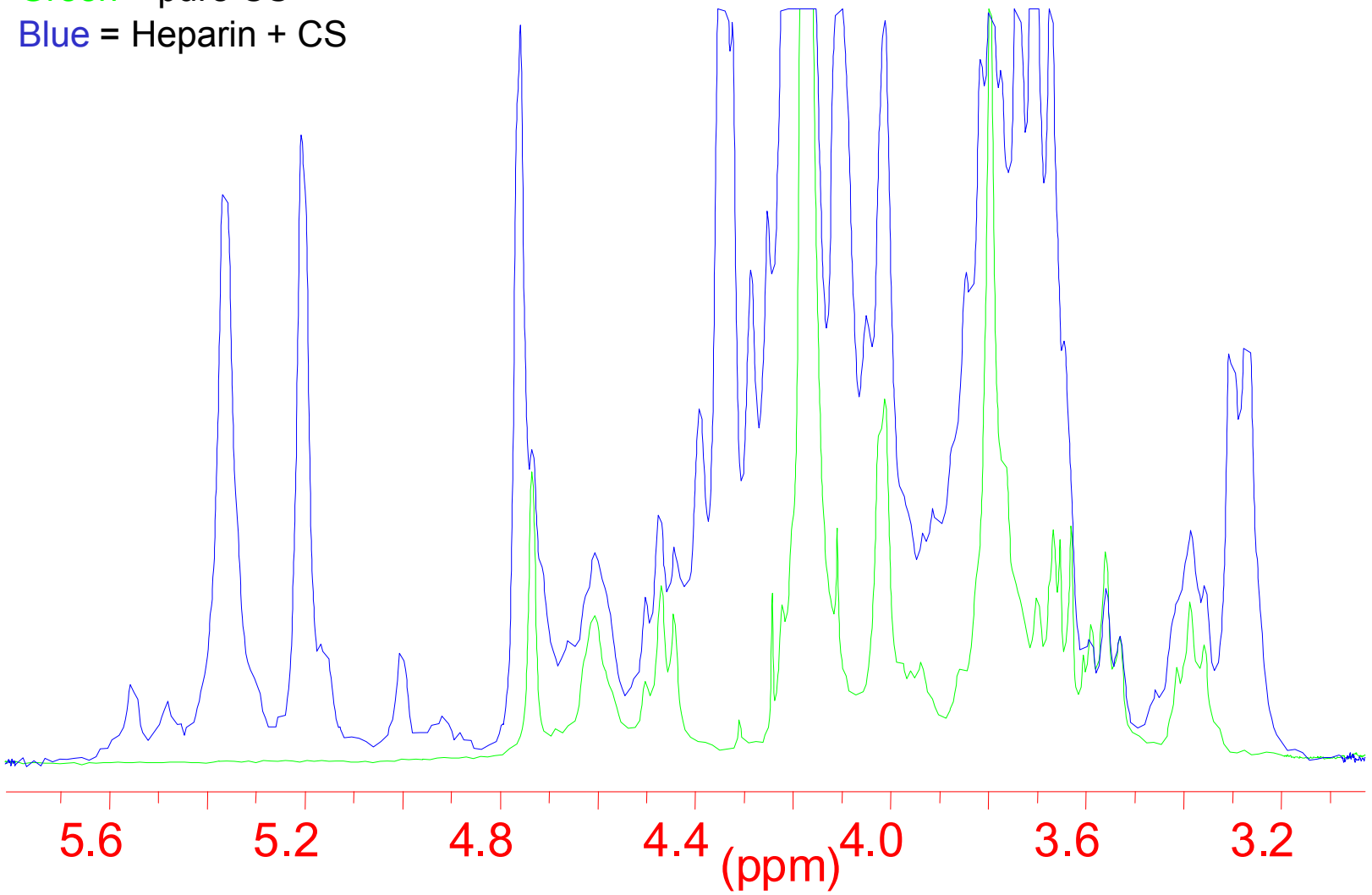
Green = pure CS
Blue = Heparin + CS



HTHNMR of Heparin spiked with CS at 353 K, detail

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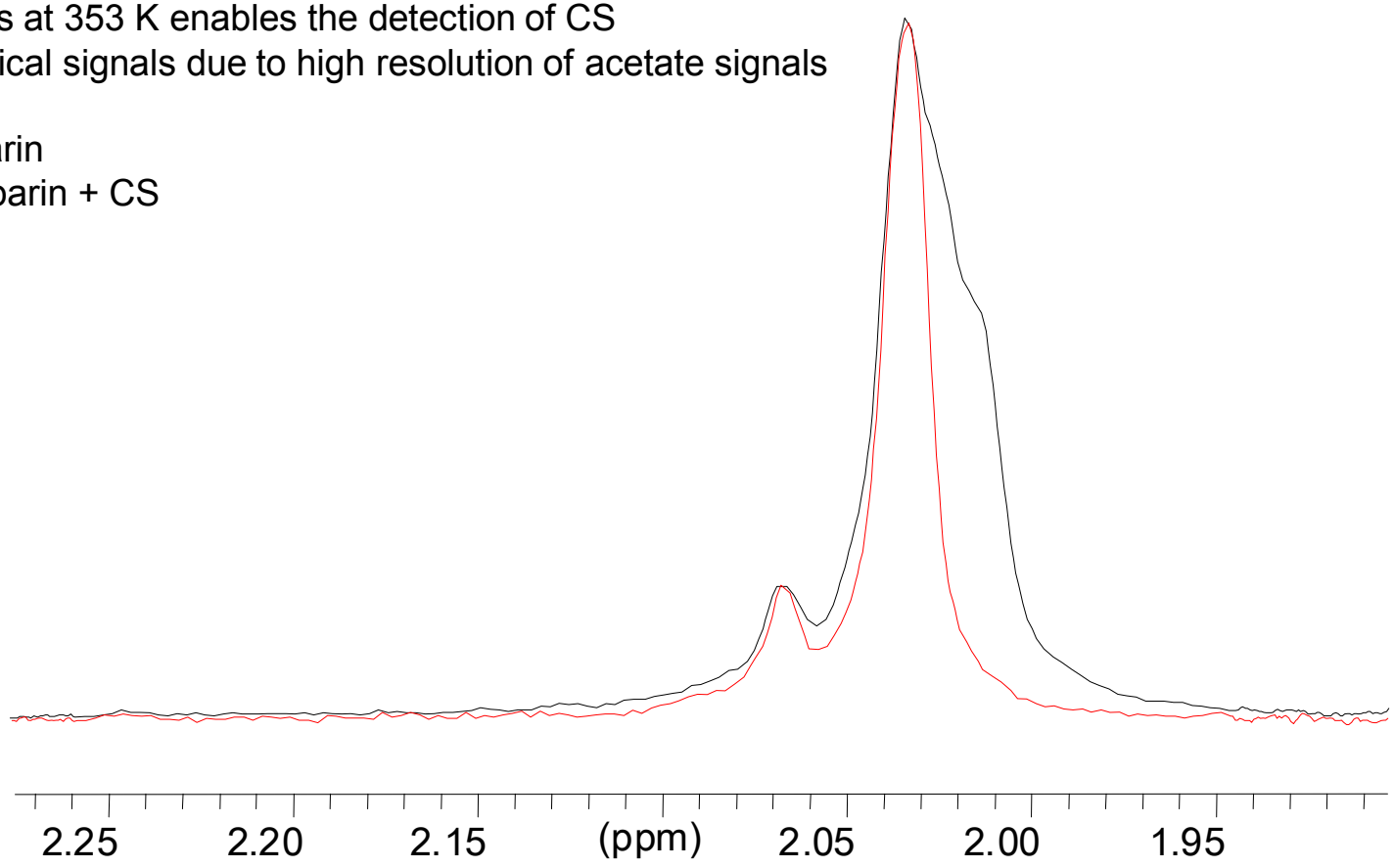
High resolution of N-acetate signals

The analysis at 353 K enables the detection of CS characteristical signals due to high resolution of acetate signals

Red = Heparin
Black = Heparin + CS



HTHNMR of Heparin spiked with CS at 353 K, detail

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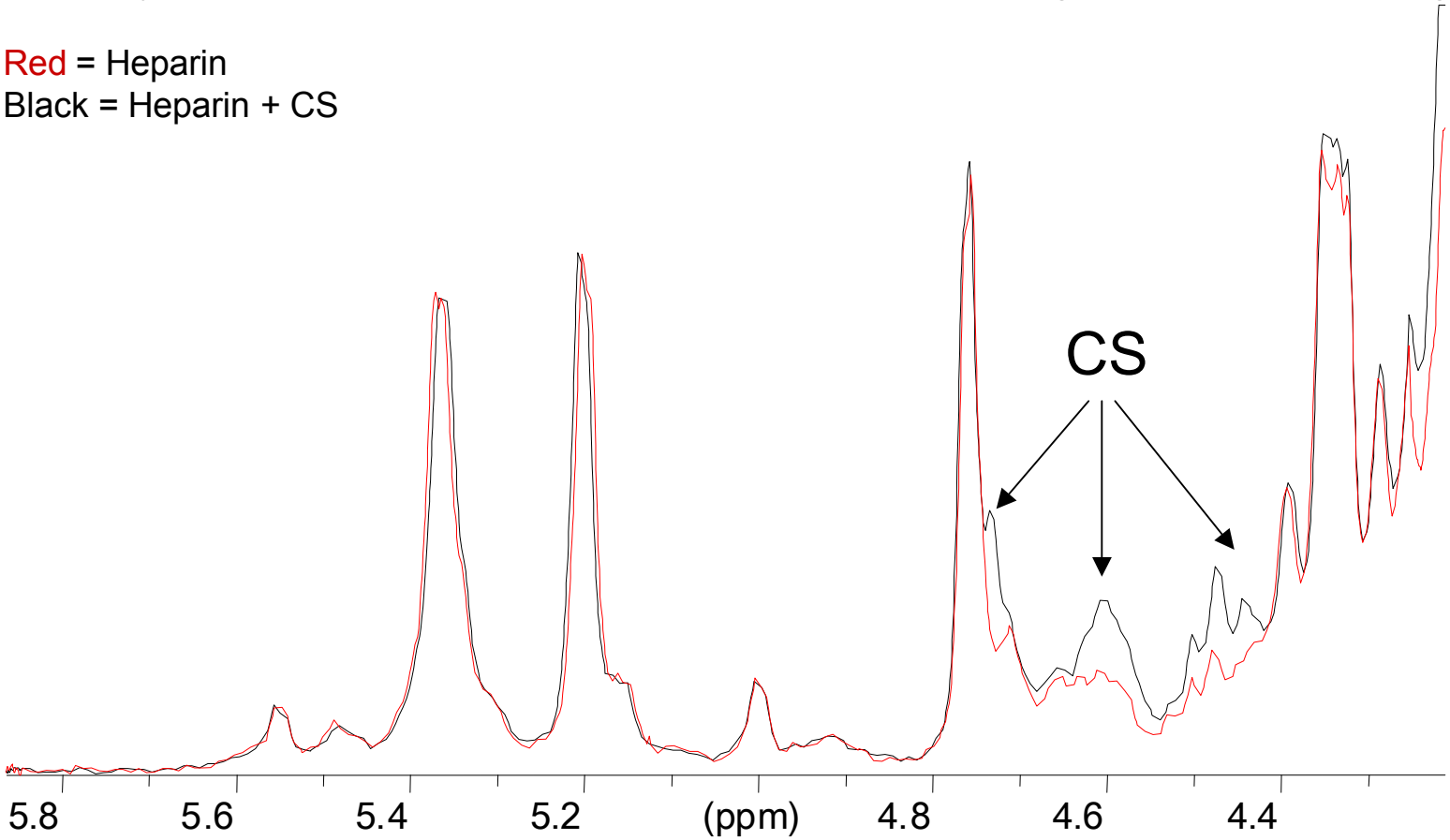
High resolution of anomeric signals

The analysis at 353 K enables the detection of CS characteristical signals due to the water signal shift.

Red = Heparin
Black = Heparin + CS



Conclusion:

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Adulteration of Heparin by OSCS and by-products like dermatan sulfate are detectable by ^{1}H NMR spectroscopy at room temperature. 300 MHz is a sufficient field strength.

Solvent residues like methanol and ethanol can be quantified simultaneously.

^{1}H-NMR spectroscopy at high temperature (353 K) is necessary for the detection of CS adulteration. Since HTNMR is a common method in NMR laboratories it should be preferred.

2-Dimensional and ^{13}C NMR are useful, but higher field strengths respectively a cryo probe is recommended.

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All analysis available

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- performed at 300 MHz or 600 MHz device.
- **within 48 hours** after arriving of sample.

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