Science with Passion

magritek Benchtop NMR Spectroscopy

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Analysis of HPLC fractions by Benchtop NMR

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SUMMARY

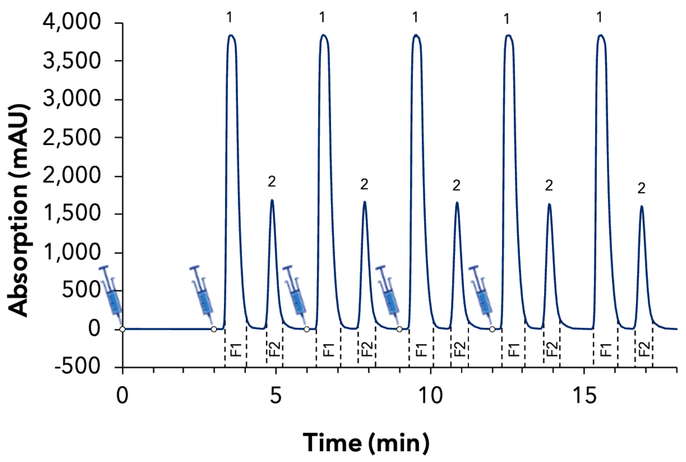
To show the capabilities of preparative HPLC combined with fraction analysis by benchtop NMR, a pharmaceutical sample was purified. The identity of the target substance was subsequently confirmed by NMR. The fractions with a concentration range of 0.7 to 4.4 mM were measured without any sample preparation, delivering quick results for batch control. Only 300 µl of sample volume per fraction were needed for an identity confirmation with no product lost. This proof-of-concept study successfully shows that a benchtop NMR can be used as an offline .or at-line HPLC detector.

INTRODUCTION

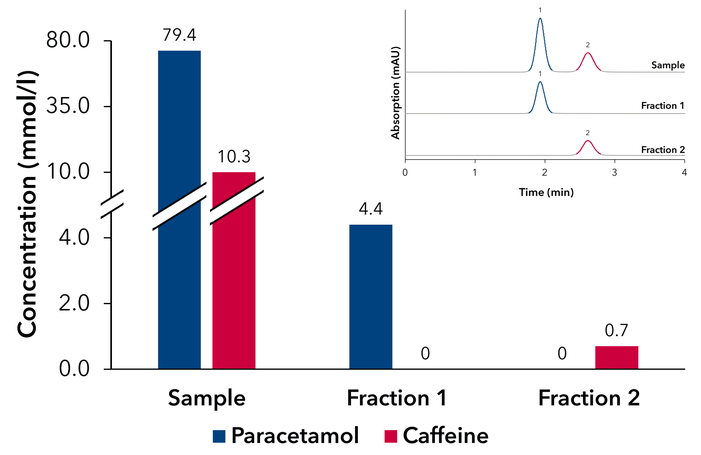
Preparative HPLC is essential for isolating compounds and removing impurities in pharmaceutical and chemical industries. Non-destructive, fast analysis is preferred to maintain yield and enable at-line monitoring, avoiding lengthy sample preparation. Recent advancements in benchtop NMR spectrometers (up to 90 MHz) allow quick analysis without deuterated solvents. In this proof-of-concept study we purified a pharmaceutical sample via HPLC and confirmed its identity using benchtop NMR. Fractions (0.7–4.4 mM) were analyzed without preparation, using only 300 µL per sample, ensuring rapid batch control with no product loss. This study demonstrates the seamless integration of HPLC and benchtop NMR.

RESULTS

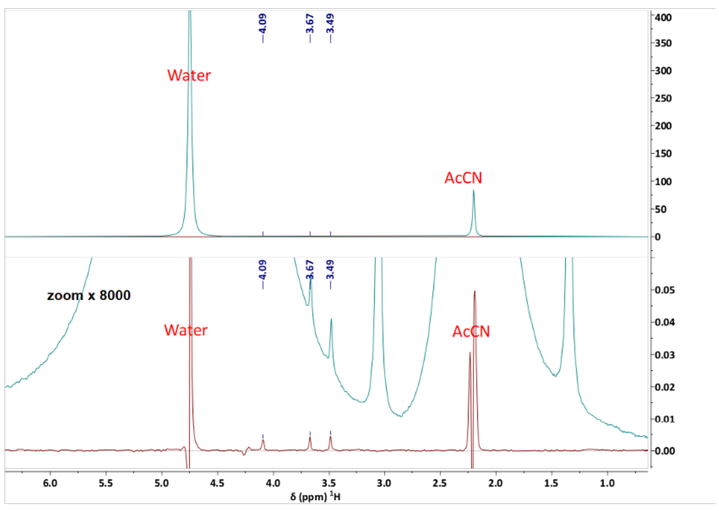
For a proof-of-concept study, a sample of paracetamol and caffeine pharmaceutical standards was purified using a KNAUER Azura® semi-preparative system in stacked injection mode. The compound mixture was separated, and repetitive fractions were pooled automatically. The collected fractions, dissolved in an ethanol/water mixture (a typical HPLC solvent), were analyzed using a Magritek Spinsolve 80 ULTRA benchtop NMR. As this device does not require deuterated solvents, the identity of both compounds was confirmed with volumes as low as 200 µl per fraction. Powerful solvent suppression eliminated sample preparation for a direct analysis. Since the measurement is non-destructive, all samples were recoverable.



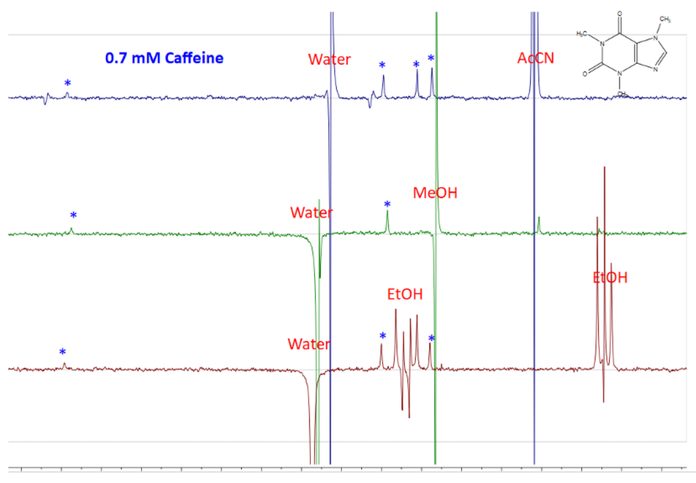
Purification of (1) Paracetamol and (2) Caffeine; Stacked injections with 500 µl injection volume; fractions 1 and 2 (F1 & F2) were pooled automatically



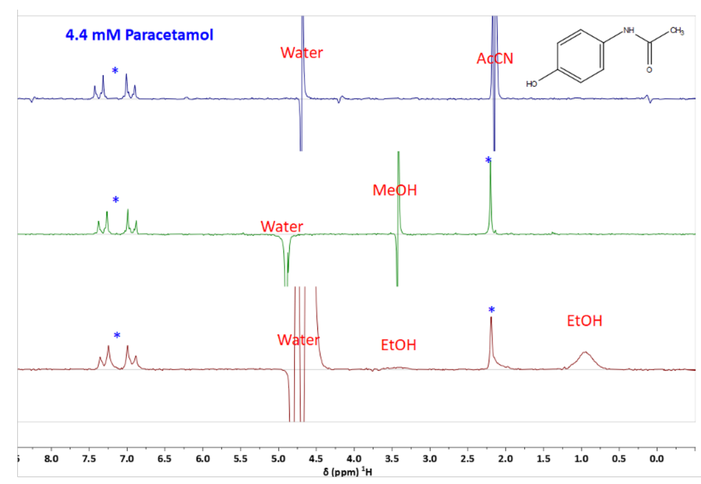
Fraction analysis with analytical HPLC system; (1) Paracetamol and (2) Caffeine; Sample was diluted 1:10; Fractions were injected directly; Recovery was 95% for paracetamol and 91% for caffeine



Comparison of ^{1}H NMR spectra of 0.7 mM caffeine in 20:80 methanol / water without (green) and with (brown) WET solvent suppression. The top panel shows the solvent signals at full scale, while the bottom one depicts a 8000x vertical zoom to show the effectiveness of the solvent suppression.



^{1}H NMR spectra of 0.7 mM caffeine with WET solvent suppression in three solvent mixes, 20:80 acetonitrile / water (top), 20:80 methanol / water (middle) and 20:80 ethanol / water (bottom). The signals of caffeine are marked by a blue asterisk. Measurement time was 9 min.



^{1}H NMR spectra of 4.4 mM paracetamol with WET solvent suppression in three solvent mixes, 20:80 acetonitrile / water (top), 20:80 methanol / water (middle) and 20:80 ethanol / water (bottom). The signals of paracetamol are marked by a blue asterisk. Measurement time was 9 min.

CONCLUSION

This proof-of-concept study successfully shows that HPLC and benchtop NMR can be used together easily. Samples can be analyzed non-destructively within minutes and without any further preparation due to highly efficient suppression of multiple solvent signals. Compounds can be identified without the need of reference standards. This study shows that NMR can provide a valid compatible alternative detector for HPLC fraction analysis.

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MATERIALS AND METHODS

Preparative HPLC Method:
Eluent 20:80 Ethanol/water, isocratic mode, flow rate 12.5 ml/min, detection UV254 nm 2 Hz, 500 µl stacked injection, automated fractionation with threshold function, column temperature ambient, Column Eurospher II 100-10 C18 150x20 mm

Analytical HPLC Method:
Eluent 20:80 Ethanol /water + 0.1% formic acid, isocratic mode, flow rate 1 ml/min, detection UV254 nm 10 Hz, 5 µl partial loop injection, column temperature 25 °C, Column Eurospher II 100-5 C18 150x4 mm

NMR Method
NMR spectra were acquired on a Magritek Multi-X Ultra 80 MHz benchtop NMR system using an adapted WET sequence for solvent suppression. 32 scans were acquired with a repetition time of 15s. WET parameters were adjusted for each sample to yield optimal suppression for all solvent signals.
Pooled HPLC fractions were analysed directly without addition of deuterated solvents or other sample preparation.

Azura® Semi Preparative HPLC
System ready for stacked injections with P 6.1L binary HPG pump 50 ml/min, AS 6.1L autosampler, UVD 2.1L UV-detector and FC 6.1 Fraction Collector

Magritek Spinsolve 80 Multi-X Ultra Benchtop NMR System
80 MHz benchtop NMR system with ultra high resolution (linewidth 50% ≤ 0.2 Hz, 0.55% ≤ 8 Hz, 0.11% ≤ 16 Hz), equipped with a 20-position autosampler