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High-Resolution Capillary Tube NMR. A Miniaturized 5-μL High-Sensitivity TXI Probe for Mass-Limited Samples, Off-Line LC NMR, and HT NMR

Götz Schlotterbeck,*,† Alfred Ross,† Remo Hochstrasser,† Hans Senn,*,† Till Kühn,‡ Daniel Marek,‡ and Oscar Schett‡

Pharma Research Basel, F. Hoffmann-La Roche AG, CH-4070-Basel, Switzerland, and Bruker Biospin AG, Industriestrasse, CH-8117 Fällanden, Switzerland

A new triple-resonance (TXI) (^{1}H, ^{13}C, ^{15}N) high-resolution nuclear magnetic resonance (NMR) capillary probe with 2.5-μL NMR-active sample volume (V_{obs}) was built and tested for applications with mass- and volume-limited samples and for coupling of microbore liquid chromatography to NMR. This is the first microliter probe with optimized coil geometry for use with individual capillary tubes with an outer diameter of 1 mm. The 90° pulse lengths of the 1-mm microliter probe were below 2 μs for proton, below 8 μs for carbon, and below 20 μs for nitrogen, and a spectral line width at signal half-height below 1 Hz was obtained. Compared to a conventional 5-mm probe, the new 600-MHz 1-mm TXI microliter probe with *z*-gradient shows an increase in mass sensitivity by a factor of 5, corresponding to a 25-fold reduction in measuring time. The consumption of costly deuterated solvent is reduced by at least 2 orders of magnitude. The 1-mm TXI microliter probe with *z*-gradient allows the measurement of one-dimensional ^{1}H NMR and two-dimensional heteronuclear NMR spectra with a few nanomoles (micrograms) of compound with high sensitivity, speed, and quality. This is a breakthrough for discrete sample NMR spectroscopy with paramount importance for structure elucidation in natural compound chemistry and metabolic research. It offers also advantages for linking chromatographic methods to NMR in an industrial environment. Capillary tube NMR may find new applications in areas where high sample throughput is essential, e.g., in the quality control of large sample arrays from parallel chemistry, screening, and compound depositories. It has the potential to increase the sample throughput by 1 order of magnitude or more if new hardware for fast sample handling and exchange becomes available.

The geometry of the sample container for high-resolution nuclear magnetic resonance (NMR) spectroscopy has remained essentially unchanged since the early days of NMR application in analytical chemistry in the 1960s. Today the conventional NMR sample still consists of a cylindrical glass tube of 5-mm outer diameter and ∼20-cm length with a sample fill volume of 500−600 μL.

Historically, the need for relatively large amounts of sample was dictated by the low sensitivity of the NMR instrumentation available in the 1960s and 1970s. Since then, the availability of superconducting magnets with higher field strength and the steady improvement of the NMR probe have increased the sensitivity of the method.[1] Today, ultra-high-field NMR spectrometers up to 900 MHz and cryogenically cooled detector coils are available. Nevertheless, structural analysis of mass-limited samples in the microgram range, e.g., natural product isolates, drug metabolites, or fractionated peaks from micro- or capillary-HPLC, still remains challenging. With decreasing diameter of the NMR detector coil, the NMR mass sensitivity (S/N per mole)[2] increases with $1/d$ to a first approximation.[3] The development of 3-[4,5] and 2.5-mm micro-NMR probes (Bruker Biospin) with sample volumes of 150 and 100 μL, respectively, was reported in 1992. In 1993, a magic angle spinning liquid nano-NMR probe (Varian) with 40-μL sample volume was introduced.[6,7] More recently a 1.7-mm submicroprobe with 30-μL fill volume was developed (Nalorac).[8]

An additional advantage of these low-volume probes is that signals from solvent impurities are much less prominent with decreasing sample volume. For electrically conductive solvents—such as salt-containing solutions—there is also a reduction of the "solvent noise" when going to smaller volumes.[9] An other advantage of small-volume NMR probes is that the amount of expensive deuterated solvents can be dramatically reduced (often by 2 orders of magnitude).

* Corresponding authors. Fax: ++41-(0)61-6887408. E-mail: hans.senn@roche.com. E-mail: goetz.schlotterbeck@roche.com.

† F. Hoffmann-La Roche AG.

‡ Bruker Biospin AG.

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So far, small-volume NMR probes have found application almost exclusively in the structural analysis of mass-limited samples.[10−12]

All NMR detectors with probe volumes of ≥45 μL described in the literature[4,5,8] are based on Helmholtz[13] coils. This coil geometry allows for easy sample exchange either by flow injection or by discrete glass tubes, as the coil opening is parallel to the magnet bore. Further miniaturization of NMR detectors below the 45-μL boundary could hitherto be achieved with capillary flow probes using solenoid coils.[14−16] However, since the solenoid coil design cannot be used for a vertical sample arrangement, it intrinsically implies that its application for discrete sample tubes is not straightforward. Today, most commercial probe manufacturers typically use the solenoid coil design in a flow NMR setup.

There are several reports about small-volume probes (<10 μL) in the recent literature. All of them operate in a flow injection setup, and they are mostly used in an on-line (flow) HPLC NMR coupling. A 1-mm microbore HPLC on-line coupling to NMR was demonstrated by Wu et al.[17] with an active volume of the NMR flow cell of ∼50 nl. Although this system showed an excellent mass sensitivity (S/N per mole),[2] it suffered from rather broad NMR line widths (>10 Hz) and a severe volume mismatch between separation peak and NMR detection volumes—in their setup, the volumes of the chromatographic peaks were ∼5 μL compared to a probe volume of 50 nL.

Another way to couple microbore-HPLC with NMR was achieved by modifying Bruker Biospin 2.5- or 2.0-mm Helmholtz coil probes.[18,19] Capillary inserts to these NMR coils with flow cell volumes between 200 and 400 nL (filling factor <1%) were constructed for NMR on-flow coupling. Here line widths of as little as 1.2 Hz were reported.[20,21]

Subsequently, solenoid microcoils with improved NMR line width were constructed.[22−24] Very recently, a flow probe with 1.5-μL cell volume—based on solenoid coil geometry—has been reported for on-line micro-HPLC coupling or manual flow injection by MRM (Magnetic Resonance Microsensor, Inc.).[25,26] This flow probe has a line width of ≤1 Hz and a hump of 12/24 Hz (0.55%/0.11% peak hight), and its cell volume matches for typical capillary HPLC peak volumes.

In this publication, we report on miniaturized NMR applications with discrete capillary sample tubes in pharmaceutical research and quality control using a new 1-mm triple-resonance (TXI) (^{1}H, ^{13}C, ^{15}N) microliter probe with *z*-gradient and 2.5-μL active sample volume. It is the first capillary probe with a vertical coil design and thus allows for easy manual sample exchange or sample exchange by commercially available automation equipment.

Using a single glass capillary tube for each individual sample is an alternative to the flow injection method in several areas of analytical NMR such as the following:[27,28] (1) the structural characterization of mass- and volume-limited samples; (2) coupling of microbore HPLC to NMR, and (3) applications where very high sample throughput (HT NMR) is essential but only a limited sample volume is available. This latter case is of growing importance, as there is an increasing demand for quality control everywhere substance libraries are used. New, sensitive, and fast NMR methods are urgently needed to meet these analytical demands in our industry.

EXPERIMENTAL SECTION

Chemicals. Deuterated solvents were purchased from Cambridge Isotope Laboratories (CIL; Innerberg, Switzerland) (acetonitrile-d_3 99%, D_2O 99.9%) and Merck AG (Darmstadt, Germany) (acetone-d_6 99.8%). Acetonitrile (Chromasolv) and water (Chromasolv) for chromatography were purchased form Merck AG. ibuprofen was purchased from Aldrich AG (Steinheim, Germany), boldine from Sigma AG (Steinheim, Germany), and strychnine from Fluka AG (Buchs, Switzerland).

Capillary Tubes. Glass capillary tubes of 100-mm length with an outer diameter of 1 mm and an inner diameter of 0.8 mm were used (Hilgenberg GmbH, Malsfeld, Germany).

Microbore Separation. The HPLC system consisted of a Agilent 1100 binary pump, a Bruker photodiode array detector with an LC Packings U−Z View flow cell of 140-nL volume, and an LC Packings 1.0 mm × 150 mm C8 column. The separation was monitored at 280 nm. A 1-μg sample of boldine was injected. The chromatographic separation was carried out with an isocratic solvent system of 70/30 (v/v) acetonitrile/water at a flow rate of 50 μL/min. Chromatographic peaks were collected using a Probot robot (BAI GmbH, Lautertal, Germany) either in capillaries or on a well plate.

Preparation and Handling of Small-Volume Samples for the Probe. Sample handling and sample exchange for the 1-mm microliter probe are similar to those for the standard NMR setup using 5-mm tubes. For mass-limited samples, we use commercially available spinners which vary slightly from the 5-mm sample tube spinners as they have been adapted to carry the 1-mm capillaries as shown in Figure 1. The spinner can then be inserted into the magnet either manually or by the conventional automatic sample

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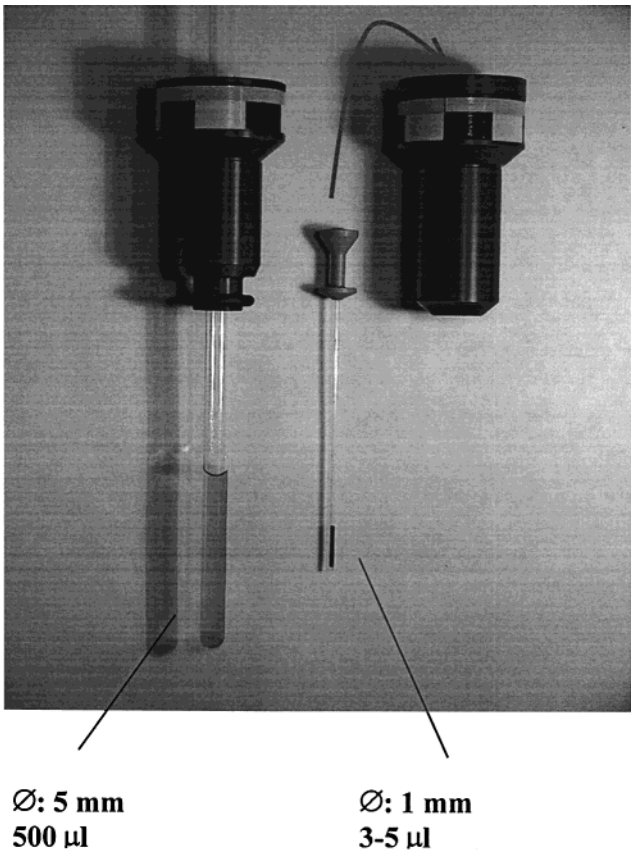

Figure 1. A 1-mm sample capillary equipped with an adapter and a modified (commercially available) Bruker Biospin spinner to carry the capillary (right). For comparison, the conventional 5-mm NMR sample tube is also shown.

changing system using the existing pneumatic sample lift. The 1-mm capillaries can either be filled manually with a syringe or by using a pipetting robot. The funnel-type capillary holder (compare Figure 1) guides the dispensing needle of the pipetting robot or a syringe into the capillary. An automatic capillary-filling robot has been developed by Roche for transferring samples from standard 96- or 384-well plates into the 1-mm NMR capillaries. At present, the robot works with six needles in parallel. The capillary sample format is fully compatible with the standardized microtiter plate format used in the pharmaceutical industry, e.g., for biological screening. This makes it intrinsically faster than the commercially available 1-mm sample filling robot from Bruker Biospin, which also works with the standard well plate format but uses only one needle.

NMR Spectroscopy. NMR spectra were recorded on a Bruker Biospin DMX 600 spectrometer equipped with the 1-mm TXI microliter probe with *z*-gradient. The active volume of the probe is ~2.5 μL. The nonspinning line shape test resulted in a line width of ≤1 Hz and a hump of 15 and 25 Hz at 0.55% and 0.11% peak height, respectively. Measurements were carried out without spinning at 300 K. All data were acquired using digital filtering.

1D Proton Spectra of 172 µg of *Sucrose in 5 or 550 µL of D_2O, Respectively.* A total of 32K data points were recorded with a spectral width of 4006 Hz, in an acquisition time of 4.1 s with a single transient. The data were processed by applying an exponential window function with 1 Hz line broadening prior to Fourier transformation.

1D Proton Spectrum of 5 µL of an 8 mM DMSO-d_6 Solution of a Screening Hit. Eight transients were collected into 32k data points using an 1D NOESY presaturation[29] pulse sequence with presaturation of the water signal during the relaxation delay of 1 s and the mixing period of 100 ms. A spectral width of 8389.26 Hz with an acquisition time of 1.95 s/scan were used, resulting in a total acquisition time of 41 s including four dummy scans. The data were processed by application of an exponential window function with 0.3 Hz line broadening prior to Fourier transformation (FT).

1D Proton Spectrum of 1 µg of Ibuprofen in 5 µL of DMSO-d_6. A total of 32K data points were recorded with a spectral width of 7184 Hz, in an acquisition time of 2.3 s. A relaxation delay of 2 s was used. A total of 16 transients were added with a total acquisition time of 70 s. An exponential window function with 0.3 Hz line broadening was applied before the Fourier transformation step.

1D Proton Spectra of Solutions of 4 µL of Rat Plasma or Cerebrospinal Fluid (CSF), Respectively, in 1−2 µL of D_2O. Rat plasma was recorded with a CPMG[30] pulse sequence. A total of 32K data points were acquired with a spectral width of 12 020 Hz, in an acquisition time of 1.38 s. A total spin−echo time of 80 ms (80 blocks) was used for the CPMG sequence. The water resonance was saturated by selective irradiation during the relaxation delay of 3 s. A total of 256 transients were added within 19 min. The spectrum was processed with an exponential window function of 1 Hz line broadening prior to FT. CSF spectra were recorded with a 1D NOESY presaturation pulse sequence with presaturation of the water signal during the relaxation delay of 1 s and the mixing period of 100 ms. 1K transients with 32K data points and an acquisition time of 2.7 s were recorded within 78 min, spectral width 6127 Hz. Before FT, exponential multiplication with a line broadening of 0.3 Hz was applied to the FID.

1D Proton Spectrum of a HPLC Peak after Injection of 1 µg of Boldine on the Column. The spectrum was recorded on a HPLC peak with ~7-µL volume and acquired with a spectral width of 6009 Hz, in an acquisition time of 2.7 s. During the relaxation of 2 s, the water resonance was saturated by selective low-power irradiation. A total of 128 transients were accumulated within 14 min. The data were processed as described above.

$^1H^{13}C$ gHSQC on 1 µg of Ibuprofen in 5 µL of DMSO-d_6. A standard sensitivity-enhanced gradient inverse-detection HSQC pulse sequence was employed.[31] During acquisition, broad-band GARP[32] decoupling was applied. The delay for the one-bond coupling was set to 165 Hz. A total of 160 t1 increments with 256 transients and 4K complex data points were acquired with a spectral width of 7184 Hz in direct dimension and 21128 Hz in the indirect dimension, respectively. The total acquisition time amounts to 20 h. The data were treated with a shifted (π/2) squared sine bell window function in both dimensions and zero-filled in the indirect dimension to 1024 data points before 2D FT.

$^1H^{15}N$ Heteronuclear Shift Correlation Spectrum (gHMBC) on 334 µg of Strychnine in 5 µL of DMSO-d_6. A standard pulse

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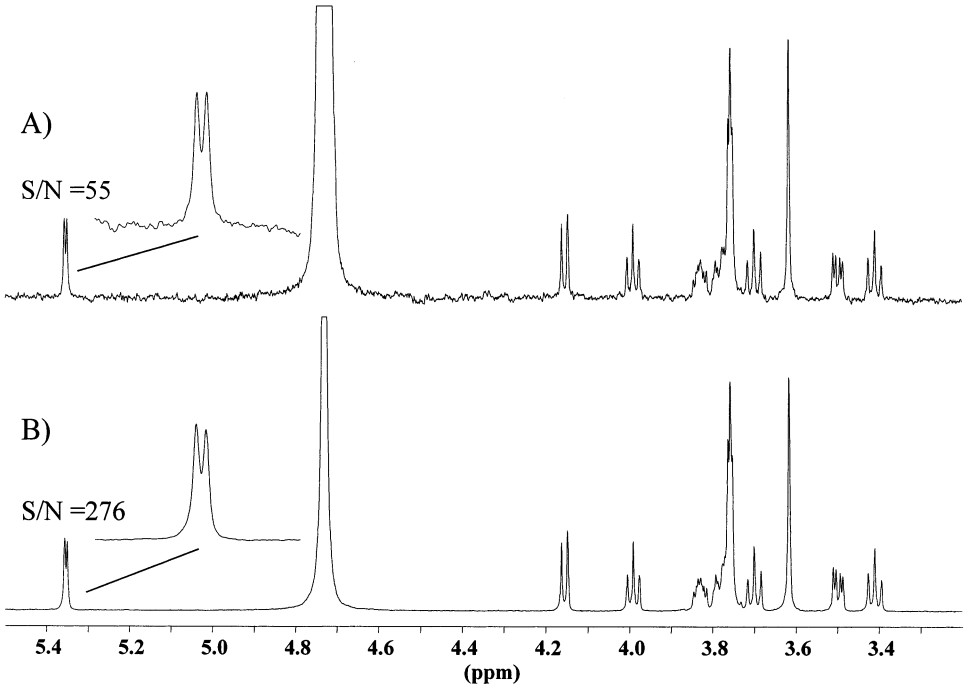

Figure 2. Comparison of ^{1}H NMR spectra of 500 nmol of sucrose (172 μg) in D_2O recorded with one transient at 600 MHz (300 K). (A) Dissolved in 550 μL in a conventional 5-mm NMR tube, obtained S/N = 55. (B) Dissolved in 5 μL in the new 1-mm microliter probe, obtained S/N = 276.

Table 1. Comparison of Probe Performance of a 1-mm TXI Microliter Probe, a 5-mm TXI Probe, and a 5-mm Cryoprobe[a]

probe	fill volume V_{tot} (μL)	Akt volume V_{obs} (μL)	reported S/N of 172 μg of succrose (0.5 μmol) in V_{tot}, NS = 1, LB = 1	S/N/μmol in V_{obs}	enhancement
1-mm TXI microliter probe	5	2.5	276	1104	5.0
5-mm conventional TXI	550	278	55	220	1.0
5-mm TXI cryoprobe	500	278	181	651	3.0
5-mm TXI cryoprobe with 1-mm capillary	22	10	195	858	3.9

[a] The obtained sensitivity (determined by the Bruker Biospin software routine) was normalized to the signal-to-noise ratio of 1 μmol of samples in the NMR active volume (column 5). Enhancement factors were given relative to the conventional 5-mm probe. All measurements were performed at 600 MHz.

program for the HMBC experiment with gradient selection was used. It uses a low-pass filter to suppress one-bond interactions, and it was optimized for long-range couplings. The delay for the evolution of the long-range couplings was set to 100 ms, and the spectrum was acquired with 6144 data points in the direct dimension and 128 data points in the indirect dimension. The spectral width for the proton dimension was 4496 and 21 131 Hz in the ^{15}N dimension. The transmitter frequency offsets were set to 4.635 and 100 ppm for ^{1}H and ^{15}N, respectively. The total experiment time was 2 h, 6 h, or 2 days, respectively. The resulting data were treated with a squared cosine window function and processed using 2-fold zero-filling in the indirect dimension before 2D FT.

RESULTS AND DISCUSSION

A new triple-resonance (^{1}H, ^{13}C, ^{15}N) high-resolution probe with 2.5-μL NMR-active sample volume (V_{obs}) was built and tested for applications with discrete mass- and volume-limited samples. The 90° pulse lengths were below 2 μs for proton, below 8 μs for carbon, and below 20 μs for nitrogen.

The miniaturized probe was designed for a 600-MHz NMR instrument (Bruker Biospin) to increase the mass sensitivity by a factor of 5 compared to a conventional 5-mm probe. In Figure 2, the S/N obtained in single-scan ^{1}H NMR spectra of 172 μg of sucrose (500 nmol) are compared for the conventional 5-mm TXI- and the new 1-mm microliter probe. Here the inherent sensitivity of the miniaturized probe is improved by a factor of 5 as expected from the 1/coil-size dependency of the S/N value[3].

In Table 1 the mass sensitivity (S/N per mole) of the 1-mm microliter probe is compared with that of a conventional 5-mm TXI probe and that of a 5-mm cryoprobe. The 1-mm microliter probe also compares well with the CapNMR flow probe from MRM[26] with respect to line shape and mass sensitivity;[33] however, it offers an alternative sample handling procedure by using capillary tubes.

The triple-resonance (^{1}H, ^{13}C, ^{15}N) capability of the 1-mm microliter probe, together with its *z*-gradient accessory, allows for

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the first time the acquisition of state-of-the-art triple- or double-resonance experiments in the microliter-volume scale, such as heteronuclear shift-correlated 1D, 2D, and 3D spectra.

The capillary sample tubes (o.d. = 1 mm, i.d. = 0.8 mm, l = 100 mm) are filled with ~5 μL of solution (Figure 1) either manually or automatically (see Experimental Section). The amount of expensive deuterated solvent consumption is thus reduced by a factor of 100 compared to conventional 5-mm NMR tubes. Therefore, this technology could also offer an alternative to the 5-mm tubes in routine NMR work where large amounts of deuterated solvents are used today. The miniaturized sample format may facilitate the automation of sample preparation in NMR laboratories. Even though in principle the sample exchange could also be achieved by a flow injection approach, the technical details for the automation of flow injection of such small discrete sample volumes are complex. Therefore, it seems much more straightforward to use an already commercially available robot with a simple syringe pump to transfer the 5-μL aliquots into the 1-mm tubes and use existing automation tools for routine NMR work with small-volume samples.

In the following, we present different applications that are specially suited for capillary tube NMR.

Application with Mass-Limited Samples. The spectra depicted in Figure 3 clearly show the power of the miniaturized probe for structure elucidation of mass-limited samples. Using the 1-mm microliter probe, we have been able to acquire $^1H^{13}C$ gHSQC 2D NMR spectra of 5 nmol (1 μg) of ibuprofen (see Figure 3a) within 20 h, and long-range $^1H^{13}C$ gHMBC spectra of 100 nmol (20 μg) of ibuprofen (data not shown) within 5 h (the weakest correlation peak in the gHMBC spectrum is visible in the contour plot at a S/N of 9.) With a conventional 5-mm probe, neither the gHSQC nor the gHMBC spectrum of ibuprofen could be obtained under these conditions (data not shown). Also a long-range $^1H^{15}N$ gHMBC spectrum of 1 μmol (334 μg) of strychnine with ^{15}N at natural abundance could be recorded in a weekend acquisition (see Figure 3b). In a comparable 6-h experiment, most of the signals were visible, and in a 2-h experiment the chemical shifts of the two nitrogens in strychnine are clearly visible in the $^1H^{15}N$ gHMBC spectrum (data not shown). These results show that the triple-resonance (^1H, ^{13}C, ^{15}N) 1-mm microliter probe with z-gradient allows the measurement of 2D heteronuclear NMR experiments of compounds in the low-microgram range.

Application in Metabonomics and Biomarker Identification. The 1-mm microliter probe also opens new avenues in pharmacological and toxicological studies of body fluids[34] (e.g., urine, blood plasma, cerebrospinal fluid (CSF), lacrimal fluid, and saliva) in cases where only a very limited volume of the biofluid is available. This has, for example, important implications for metabonomic[35] studies with CSF of small animals (e.g., mice). Sample taking can now be carried out without sacrificing the animal. This allows one to follow the complex pattern of endogenous metabolites in a defined body fluid compartment at different time points after treatment in the same animal. As a consequence, a higher statistical significance is achieved, as the animal serves as its own control. Panels a and b of Figure 4 show the ^1H NMR spectra of 5 μL of rat CSF and rat plasma, respectively.

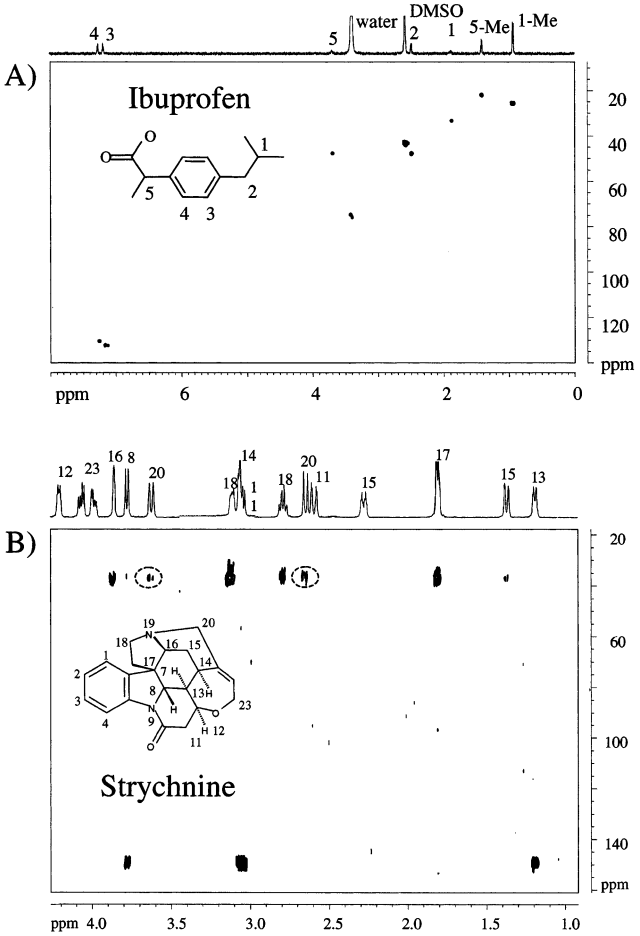

Figure 3. (A) $^1H^{13}C$ gHSQC spectrum of 5 nmol (1 μg) of ibuprofen in 5 μL of DMSO-d_6 recorded in 20 h in the 1-mm microliter probe together with a ^1H NMR spectrum of 5 nmol of ibuprofen recorded with 16 transients (~1 min). (B) $^1H^{15}N$ gHMBC spectrum of 1 μmol (334 μg) of stychnine in 5 μL of DMSO-d_6 recorded in 2 days in the 1-mm microliter probe. All signals beside the marked ones were already visible in a $^1H^{15}N$ gHMBC experiment acquired in 6 h.

Off-Line Microbore LC NMR Coupling. The high separation efficiency of microbore chromatography, and its low solvent consumption, are ideal properties to be combined with the power of miniaturized probe head technology for NMR structure elucidation. The miniaturized method allows the use of fully deuterated solvents for the chromatographic separation. In the past, miniaturized separation techniques were successfully on-line coupled to NMR, e.g., capillary HPLC,[18−20,36] capillary electrochromatography (CEC),[37,38] or capillary electrophoresis (CE).[14,15,27]

The limited sensitivity of the NMR detector, however, often requires that on-line LC NMR is performed in the "stopped-flow" mode. As NMR and HPLC operate on different time scales, it can be preferable to collect the HPLC peaks first and then analyze them one by one in the NMR probe. This "off-line" coupling

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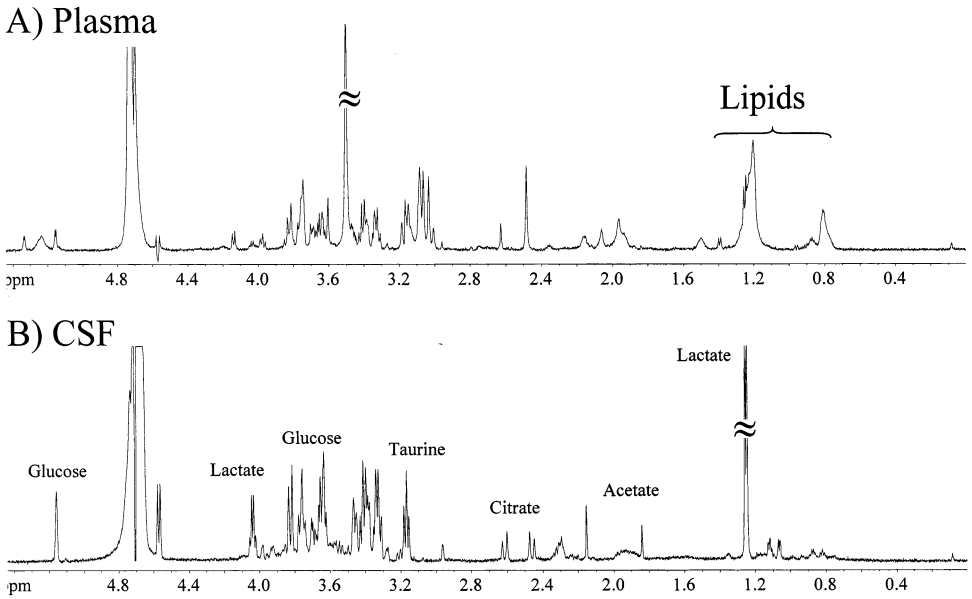

Figure 4. ^{1}H NMR spectrum of 5 μL of (A) rat plasma and (B) rat CSF, both with ~30% D_2O added.

strategy significantly widens the benefit and flexibility of LC NMR in an industrial environment. It takes into account the work flow in pharmaceutical industry where many chromatographic laboratories want to take advantage of LC NMR. As a further advantage, project-related expertise of sample separation has not to be transferred from different departments to the NMR laboratory.

For the off-line peak storage, several devices have been reported such as peak storage loops or solid-phase extraction cartridges. These two methods traditionally operate with flow injection NMR probes.

An alternative way for this "off-line" LC NMR is to use discrete sample tubes for the peak storage. The use of a 1-mm capillary NMR tube to store microbore HPLC fractions with microliter peak follows this concept. The NMR capillaries with microfractionated LC peaks can easily be collected from the different laboratories and efficiently measured with the new probe using existing NMR automation and sample-changing routines.[39] The off-line approach offers some additional benefits: potential dead volume effects caused by long transfer capillaries are minimized, and those caused by valve switching and fluidic problems are eliminated. Thus, LC peak dilution is kept to an absolute minimum, which is crucial for obtaining an optimal S/N with mass-limited samples.

Microbore LC peaks can be directly fractionated into 1-mm capillaries if fully deuterated eluents are used. If the separation is carried out with protonated solvents, the peaks can be temporarily "stored" in a well plate. In this case, the protonated eluent can easily be exchanged with deuterated solvent upon lyophilization before transferring the LC peak into the 1-mm capillary. An advantage of this procedure is that the individual LC fractions can be adjusted in a solvent volume that matches the actual NMR probe volume. To prevent sample decomposition, the fractionation system can be operated under inert conditions with a N_2 or argon atmosphere. However, for some applications such as extremely air and light sensitive samples, a closed on-line system may be advantageous. The very high sensitivity of the off-line coupled microbore LC-capillary NMR method with the use of the 1-mm microliter probe is demonstrated in Figure 5.

In this experiment, 1 μg of boldine was injected and eluted over a 1-mm HPLC column. The boldine LC fraction was then collected during the chromatographic separation in a 384-well plate, either by fractionation of the LC peak into one well position or by depositing constant small volumes (microliters) of the eluent into consecutive well positions. The protonated eluent was evaporated by lyophilization, and the remaining boldine in the collecting well position was reconstituted in acetonitrile-d_3. The peak was transferred by our robot system from the 384-well plate into the 1-mm capillary and the ^{1}H NMR spectrum was acquired within 14 min.

Quality Control for Compound Libraries by HT NMR. In the Roche central compound depository, all in-house compounds for screening are stored in multiple aliquots (a few microliters) of 5 mM DMSO solutions. Aliquots are supplied from there to be tested in the various biological assays. There is a growing demand for quality control of the compounds in these depositories. Since the stored volumes are relatively small, any analytical method addressing this issue must not consume more than a few microliters of compound solution.

NMR would be an ideal analytical tool for this task as low-level impurities are easily detectable. Next to minute sample consumption, a high sample throughput is a prerequisite to use NMR as a tool for quality control of compound depositories on a routine basis (HT NMR). The throughput is dependent on the time used for NMR data acquisition (reciprocal to the square of the sensitivity of the NMR probe) and on the time spent for sample exchange.

With the 1-mm microliter probe, a ^{1}H NMR spectrum of a sample in the millimolar concentration range can be obtained within seconds in a few scans from ~5 μL of solution. Figure 6 shows a ^{1}H NMR spectrum of 5 μL (40 nmol = 12.7 μg) of such a compound solution (MW = 317.35; elementary formula $C_{20}H_{15}$-

(39) Ross, A.; Senn, H. *Drug Discovery Today* **2001**, *6*, 583−593.

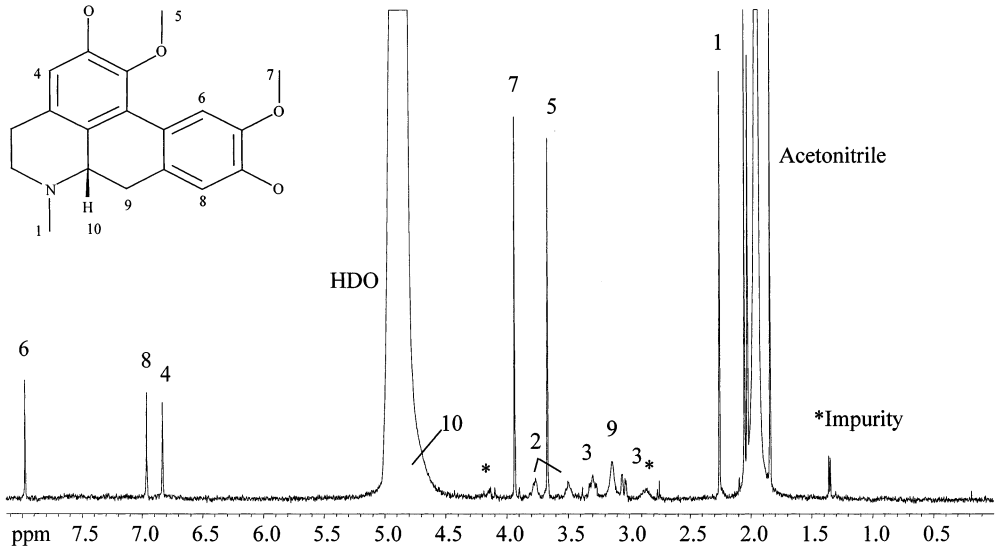

Figure 5. A 1-μg boldine sample, injected and separated over a 1-mm C8 HPLC column with protonated solvents. The fractionated LC peak (~25 μL) was reconstituted in 7 μL of ACN-d_3 and transferred to a 1-mm capillary. The ^{1}H NMR spectrum was recorded within 14 min. This experiment shows that with the 1-mm microliter probe structures of compounds may be detected and elucidated that are present in mixtures in quantities as low as 1 μg or less.

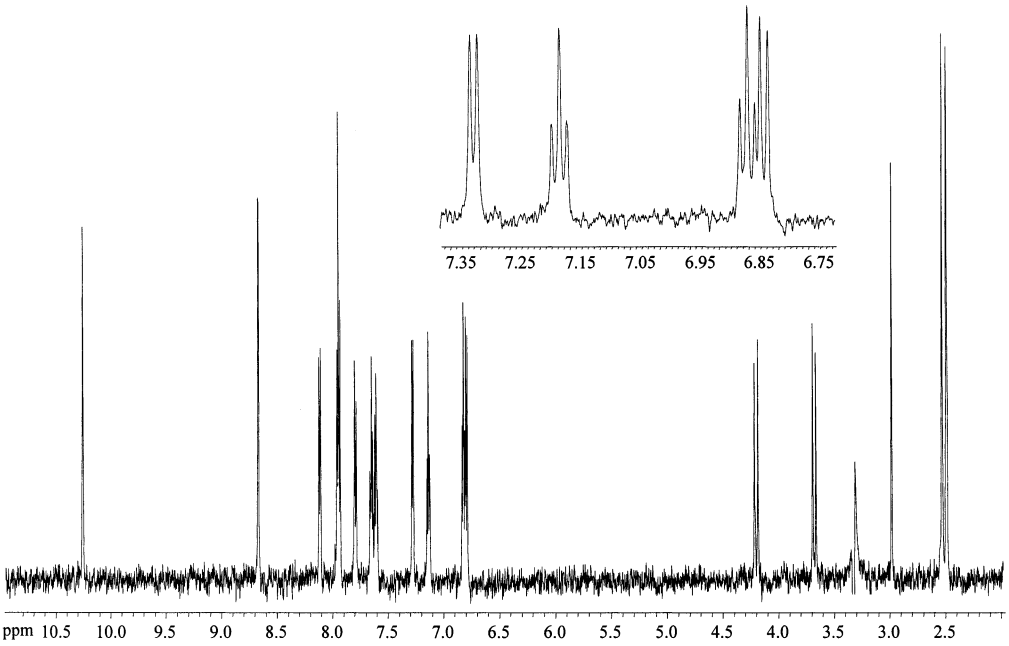

Figure 6. ^{1}H NMR spectrum of 40 nmol (12.7 μg) of a high-throughput screening hit dissolved in 5 μL of DMSO-d_6. The spectrum was acquired using the 1-mm microliter probe with eight transients in 41 s. The inset shows the excellent resolution of the spectrum obtained.

NO_3) dedicated for screening. The spectrum was obtained with eight scans within 40 s. The measurement can be carried out in an automated fashion using disposable capillaries.

With such short NMR acquisition times, the bottleneck for achieving high sample throughput using discrete samples is the time needed for sample replacement in the magnet (4−6 min).[40] Thus, the sensitivity of the NMR detection system for this application is no longer the limiting factor in sample throughput but rather the time needed for sample transfer. The sample throughput in NMR could now easily be increased by 1 order of magnitude or more if the hardware for fast sample handling and exchange were available. Thereby, miniaturization of the sample format is an essential for efficient sample handling and for placing a large number of samples in the waiting queue of the spectrometer. These requirements are ideally matched by the 1-mm capillary tubes, which can be placed in the standard 96 well plate format.

A first step toward microvolume high-throughput NMR was realized in this work with the 1-mm microliter probe in conjunction with the pipetting robot for fast replication of well plates into discrete NMR capillariy tubes. In the future, this probe may pave the way to check the purity, structure, and stability of large sample arrays from combinatorial chemistry, biological screening, or large compound depositories. Efforts to develop miniaturized, auto-

(40) Ross, A.; Schlotterbeck, G.; Senn, H.; Kienlin, M. *Angew. Chem.* **2001**, *113*, 3343−3345.

mated, and innovative technologies to meet the urgent analytical demands from pharmaceutical research are ongoing in our laboratory.

CONCLUSION

The new (^{1}H, ^{13}C, ^{15}N) high-resolution 1-mm microliter probe with *z*-gradient allows the measurement of 1D ^{1}H NMR or 2D and 3D inverse heteronuclear NMR experiments with a few nanomoles (corresponding to one to few micrograms) of compound with a hitherto unprecedented sensitivity and speed using discrete sample tubes.

This offers significant advantages for the structure elucidation of mass- and volume-limited samples, off-line microbore HPLC NMR coupling, and applications where high sample throughput (HT NMR) is essential and only a limited sample volume is available.

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