Online Coupling of Enantioselective Capillary Gas Chromatography with Proton Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT The hyphenation of enantioselective capillary gas chromatography and mass spectrometry is not always sufficient to distinguish between structural isomers, thus requiring peak identification by NMR spectroscopy. Here the first online coupling of enantioselective capillary gas chromatography with proton nuclear resonance spectroscopy is described for the unfunctionalized chiral alkane 2,4-dimethylhexane resolved on octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin at 60°C. NMR allows constitutional and configurational isomers (diastereomers and enantiomers) to be distinguished. Enantiomers display identical spectra at different retention times, which enable an indirect identification of these unfunctionalized alkanes. The presented method is still at an early development stage, and will require instrumental optimization in the future. *Chirality 22:808–812, 2010.*

KEY WORDS: chiral alkane; enantiomers; cyclodextrin; enantioselective online-coupling GC-NMR

INTRODUCTION

In chromatography, unequivocal chemical assignment requires structure elucidation of a pure compound by spectrometric and/or spectroscopic techniques such as mass spectrometry and NMR spectroscopy.[1] Unfunctionalized saturated hydrocarbons, i.e., alkanes, consist of a wide range of constitutional and configurational isomers (including enantiomers), which are difficult to distinguish by mass spectrometry. The number of constitutional and configurational isomers is dependent on the total number of carbon atoms and is increased from 24 isomers for C8 to 3395.964 for C20,[2] which requires high separation performances[3] and powerful detection systems to achieve a correct structural assignment. Due to the nonexistent functionality, the gas chromatographic enantioseparation of simple chiral alkanes cannot be achieved by hydrogen-bonding chiral selectors (e.g. Chirasil-Val)[4] or coordination-type chiral selectors (Chirasil-Metal).[5] Thus, the chromatographic separation and identification of small unfunctionalized configurational isomers represents a great challenge in separation science. Due to the absence of suitable functionalities, enantioseparation can only be achieved via weak Van der Waals forces by employing highly enantioselective selectors such as modified cyclodextrins. These selectors display unique enantioselectivities for small chiral compounds because of the possibility to include the analyte in their molecular cavities. The size of the cavities can be controlled by different substitution patterns or the number of sugar molecules in the cyclodextrin ring. The enantioseparation of configurational isomers of terpenoic cycloalkanes and cycloalkenes was for the first time solved by Kościelski in 1983[6,7] with packed columns containing Celite coated with α-cyclodextrin in formamide. With the advent of high resolution capillary gas chromatography employing permethylated ß-cyclodextrin in a semipolar polysiloxane matrix unfunctionalized 1,2-dialkylcyclohexanes could be enantioseparated in 1989[8] followed by the enantioseparation of simple chiral alkanes later on.[9–14]

Nuclear magnetic resonance (NMR) spectroscopy, which is the most powerful analytical method for the structural elucidation of organic compounds, can be envisioned as an ideal detection method to identify stereoisomers preseparated by gas chromatography. For combining the very high separation power of gas chromatography with the high information capacity of NMR spectroscopy two approaches can be found in the literature. Marriott and coworkers described quite recently two model applications for the identification of volatile compounds out of complex matrices using an offline coupling of microscale-preparative multidimensional gas chromatography (MDGC) and NMR spectroscopy.[1,15] In one of their applications they isolated 8.6 μg geraniol from a complex essential oil matrix using MDGC and recorded remarkable ^{1}H NMR spectra

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with a 800 MHz NMR spectrometer equipped with a cryoprobe within an acquisition time of 1 hr.[15] The second direct approach was published also quite recently and demonstrated the feasibility of an online coupling of gas chromatography and NMR spectroscopy[16,17] based on earlier reports of the direct combination of packed column and NMR instrumentation.[18,19] In a recent work, the successful capillary gas chromatographic separation and online identification of the stereoisomers of *E,Z*-2-pentene and of *E,Z*-2-hexene using a Chirasil-β-Dex stationary phase and a 400 MHz NMR spectrometer equipped with a custom-built, double resonant solenoidal microprobe was described.[17]

Both methods benefit from newer developments in the field of NMR such as microprobes[16,17,20,21] or cryoprobes[22–25] and stronger magnets, which dramatically improved the sensitivity of the relatively insensitive spectroscopic method so that the largest sample amounts, which can be separated by capillary GC are getting closer to the detection limit of NMR spectroscopy. Thus it is to be expected that many applications using a combined method of GC and NMR (offline as well as online) will emerge in the future especially if a high purity isolation is required for an unequivocal identification.

To the best of our knowledge, the present work reports on the first separation of enantiomers by gas chromatography with a continuous online NMR detection and it adds to the enormous potential of GC-NMR hyphenation. Moreover, the novel enantioselective online GC-NMR experiment has been probed for a most difficult enantioseparation task, i.e., that of the resolution of an unfunctionalized racemic alkane.

MATERIALS AND METHODS

Materials

Racemic 2,4-dimethylhexane was obtained from Fluka Chemie GmbH (Buchs, Switzerland). Octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G)[12] was purchased from Cyclolab Ltd. (Budapest, Hungary).

Instrumentation

NMR. All NMR spectra were recorded on a Bruker ARX 400 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). The outlet of the gas chromatographic capillary column was connected to a unheated custom-built, double resonant solenoidal microprobe with an active detection volume of 2 μl by using a 2 m fused silica transfer capillary (250 μm i.d.). For all capillary connections universal glass connectors were used (Klaus Ziemer GmbH, Langerwehe, Germany). The NMR spectrometer was operated by an O_2 workstation (Silicon Graphics) and XWIN-NMR software (Bruker Biospin GmbH, Rheinstetten, Germany). Data analysis was executed with XWIN-NMR 3.5. The 90° ^{1}H flip angle was adjusted to 10 μs (16 dB attenuation/50 W amplifier). Data acquisition parameters for stopped-flow-experiments: 60 k transients (overnight) with 10 k time domain points and a spectral width of 5580 Hz were accumulated with a relaxation delay of 500 ms. Data acquisition parameters for the 2D-experiment: For each row 64 transients with 1.5 k time domain points and a spectral width of 4595 Hz were accumulated with a relaxation delay of 10 ms. During the separation 128 rows with a acquisition time of 13.5 s/row were recorded.

Gas chromatography. The enantioseparation was performed on a Fractovap Series 2350 (Carlo Erba Strumentazione, Milan, Italy) gas chromatograph employing undiluted octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin as the stationary phase (30 m × 250 μm i.d., 0.50 μm film thickness). The carrier gas was dinitrogen 5.0 (Westfalen AG, Muenster, Germany) with a calculated flowrate of 0.5 mL/min. The oven temperature was set to 60°C during the whole separation and 0.3 μL of racemic 2,4-dimethylhexane was injected in the splitless injection mode.

Data Processing

All recorded NMR spectra show a significant background signal. To obtain more significant information, a special baseline correction procedure had to be performed. For the correction of the 1D spectra one ^{1}H NMR spectrum with exactly the same parameters but without any sample was recorded. This spectrum was subtracted from the previously recorded stopped-flow spectrum. For the correction of the rows in the 2D plots one of the first rows that show no eluting analyte was chosen. This row was subtracted from each other row in the 2D plot.

RESULTS

The gas chromatographic enantioseparation of 2,4-dimethylhexane, 2,4-dimethylheptane and 2,4-dimethyl-hep-1-ene on octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G) is shown in Figure 1. The thermodynamic Gibbs-Helmholtz parameters of enantiorecognition of 2,4-dimethylhexane by Lipodex G have been reported previously.[3] The enantioseparation factor increases from $\alpha = 1.16$ at 65°C to $\alpha = 1.53$ at 25°C.[3]

Figure 2 depicts the contour plot of the online GC-NMR enantioseparation of racemic 2,4-dimethylhexane. For this experiment a mixture of 0.3 μL racemic 2,4-dimethylhexane was injected into the gas chromatograph, which was directly connected to the NMR spectrometer.

The contour plot shows the chromatographic retention time versus the ^{1}H chemical shift axis. The flow rate can be calculated from the residence time τ of the analytes in the detection cell and its known size. The residence time τ in the detection cell can be calculated as follows:

$$W_{\text{flow}} = W_{\text{stationary}} + 1/\tau$$

$$W = \text{signal width at half-height}$$

The residence time τ in the detection cell obtained by this calculation was 0.25 s leading to a line broadening of 4 Hz and a corresponding flow rate of 0.5 mL / min.

The published enantioselectivity factor of $\alpha = 1.5$ at 30°C on octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin

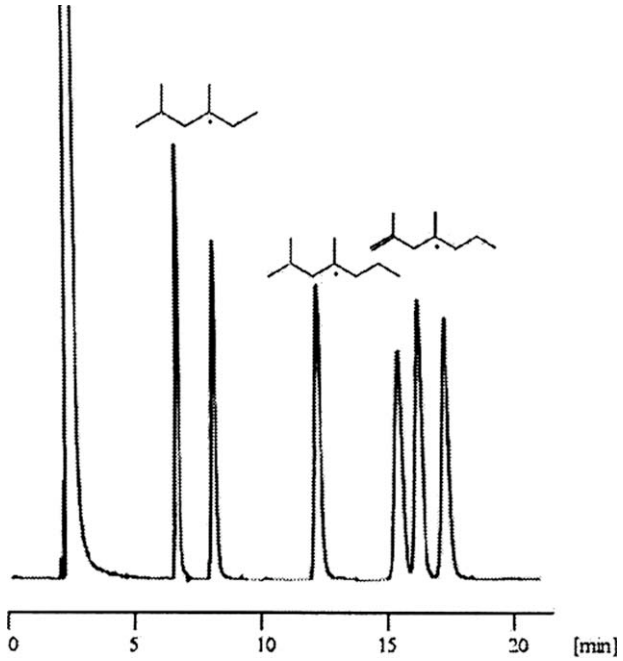

Fig. 1. Gas chromatographic enantioseparation of 2,4-dimethylhexane, 2,4-dimethylheptane, and 2,4-dimethyl-hep-1-ene on octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (Lipodex G)[12,13] (50 m x 250 μm i.d. fused silica capillary, film thickness 0.25 μm, carrier gas: 100 kPa dihydrogen, temperature: 36°C).

for the single GC experiment[3] could not be achieved for this GC-NMR setup. The reduction of the enantioselectivity is due the higher oven temperature of 60°C, which reduces the enantioselectivity of the stationary phase in the enthalpy-controlled region (below the enantioselective temperature).[26] However, the increased temperature is necessary to reach a higher concentration of the analyte in the detection volume to compensate for the insensitive NMR detection. Furthermore, the higher temperature leads to a faster separation increasing peak heights. In comparison to the uncoupled GC-FID experiment, the flow had to be reduced in the GC-NMR experiment, which decreases the resolution factor R_s due to the loss of efficency away from the van Deemter optimum. However, higher flow rates would lead to shorter residence times resulting in very broad NMR signals.

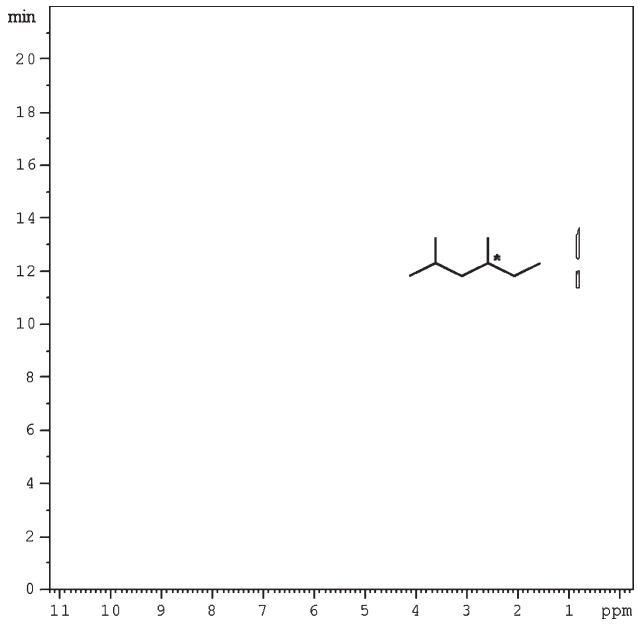

Fig. 2. Contour plot of a GC-NMR enantioseparation of racemic 2,4-dimethylhexane on octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin. Data acquisition parameters: For each row 64 transients with 1.5 k time domain points and a spectral width of 4595 Hz were accumulated with a relaxation delay of 10 ms. During the separation 128 rows with an acquisition time of 13.5 s/row were recorded.

Figure 2 is a novel proof of principle to detect the enantiomers at different chromatographic retention times. For the identification of the enantiomers the quality of the NMR spectra from the 2D-plot is not sufficient. More detailed information has to be obtained by recording stopped-flow spectra. Due to the very low concentration of the analyte in the detection cell, the background signal of the detection cell can no longer be disregarded (Fig. 3). It seems that the background signal is caused by residual protons in the glass ware of the detection cell. Also unknown contaminants, which may adhere directly to the surface of the detection cell might be responsible for the observed background noise. The problem can be solved by recording a stopped-flow spectrum of the empty detection cell, which has to be subtracted from the stopped-flow spectrum of the analyte as described in Materials and Methods. Another alternative to the differential spectra approach could be using a $T_{1\rho}$ filter to suppress the broad background signals with short relaxation times. Nevertheless the comparison of the stopped flow difference spectra of the two peaks is advanced in Figure 4. Apart from a different signal-to-noise ratio, two identical NMR spectra are

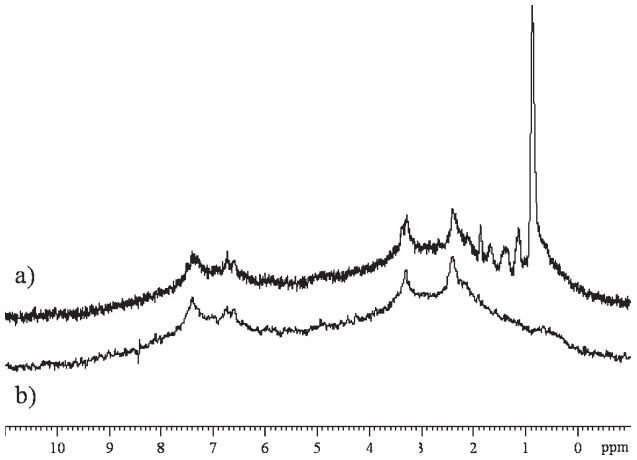

Fig. 3. Comparison of the ^{1}H NMR spectra (a) of the stopped-flow gas phase spectrum of 2,4-dimethylhexane (enantiomer of the second eluted peak) (b) of the empty GC-NMR probe with ^{1}H NMR background signals.

obtained from the two peaks which indirectly proves the presence of incongruent enantiomers.

Compared to the high resolution reference spectra it can be shown that the spectral quality of the stopped flow difference spectra are sufficient to achieve the correct structural assignment with the aid of a reference spectrum (Fig. 5), which are for many compounds commonly accessible by spectral data bases. Other helpful information such as integration results or coupling constants could not yet been obtained for this experiment due to the very low sample amount in the detection cell. Therefore an uncoupled offline system that is able to collect the different peaks of several runs would be necessary as was demonstrated by Marriott and coworkers.[1]

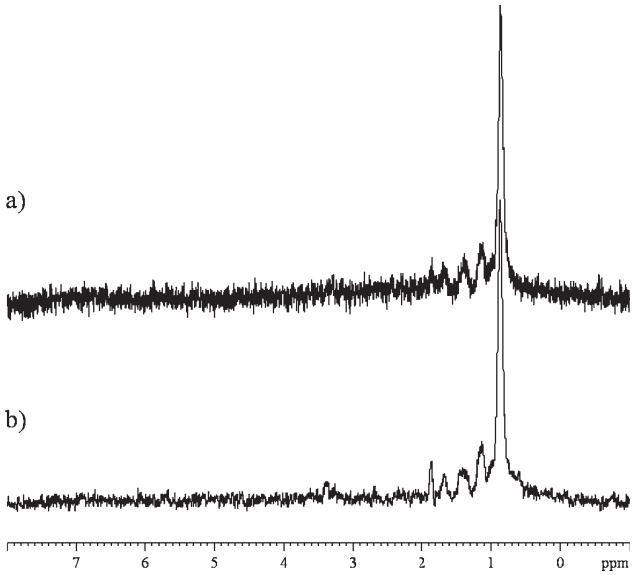

Fig. 4. Comparison of the stopped-flow gas phase difference spectra of (a) the first eluted enantiomer of 2,4-dimethylhexane (b) the second eluted enantiomer of 2,4-dimethylhexane.

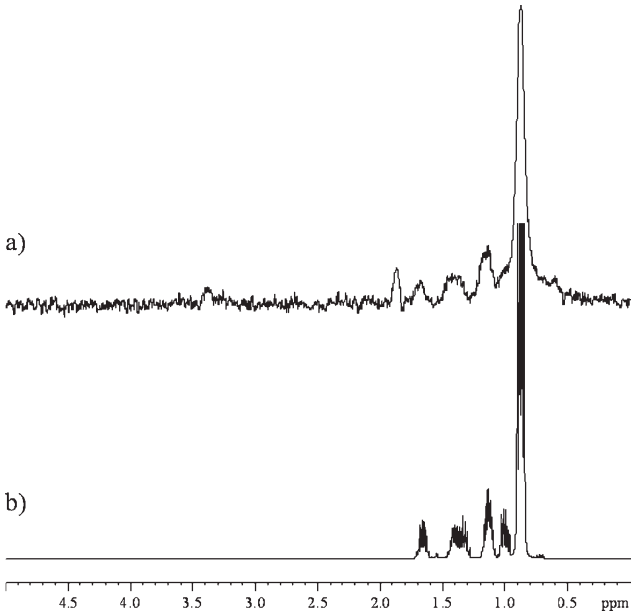

Fig. 5. Comparison of the stopped-flow gas phase ^{1}H NMR spectrum of (a) one enantiomer of 2,4-dimethylhexane and (b) the off line high resolution ^{1}H NMR reference spectrum of 2,4-dimethylhexane.

The preliminary results are promising as a combined approach employing enantio-GC and NMR methodology. Hitherto all online GC-NMR experiments have been performed at relatively low field strength of 400 MHz. The signal-to-noise ratio could be improved by a factor of four by using magnets with a proton resonance frequency of 800 MHz. Also shielded magnets would lead to a more efficient GC-NMR system due a shorter transfer line and the corresponding increased peak intensity caused by a decrease of diffusion effects.

CONCLUSIONS

In this work, it is demonstrated that even extremely challenging separation problems such as enantioseparations of small molecules can be investigated by a hyphenated GC-NMR system. Employing an online capillary GC-NMR system the successful separation and identification of the enantiomers of a volatile unfunctionalized chiral alkane via a continuous NMR detection was achieved.

Stopped-flow experiments enabled the identification of the enantiomers with the aid of reference spectra, which are for many compounds accessible by spectra data bases. The sample amount of a successful GC-NMR experiment was about 100–300 μg at 400 MHz using a solenoidal microprobe, but instrumental improvements are anticipated to further decrease this amount.

Although yet at its infancy, this first enantioselective GC-NMR experiments holds promise in the future in line with instrumental refinement. The GC-NMR approach may complement the well established GC-MS hyphenation in the realm of stereochemical analysis.

LITERATURE CITED

1. Eyres GT, Urban S, Morrison PD, Marriott PJ. Application of microscale-preparative multidimensional gas chromatography with nuclear magnetic resonance spectroscopy for identification of pure methylnaphthalenes from crude oils. J Chromatogr A 2008;1215: 168–176.
2. Ullmann Encyklopädie der Technischen Chemie. Kohlenwasserstoffe, Vol. 10: München-Berlin: Urban & Schwarzenberg; 1958. p 1.
3. Sicoli G, Kreidler D, Czesla H, Hopf H, Schurig V. Gas chromatographic enantioseparation of unfunctionalized chiral alkanes: a challenge in separation science (overview, state of the art, and perspectives). Chirality 2009;21:183–198.
4. Frank H, Nicholson G, Bayer E. Rapid gas chromatographic separation of amino acid enantiomers with a novel chiral stationary phase. J Chromatogr Sci 1977;15:174–176.
5. Schurig V. Review, Practice and theory of enantioselective complexation gas chromatograph. J Chromatogr A 2002;965:315–356.
6. Kościelski T, Sybilska D, Jurczak J. Separation of α- and β-pinene into enantiomers in gas-liquid chromatography systems via α-cyclodextrin inclusion complexes. J Chromatogr 1983;280:131–134.
7. Kościelski T, Sybilska D, Jurczak J. New chromatographic method for the determination of the enantiomeric purity of terpenoic hydrocarbons. J Chromatogr 1986;364:299–303.
8. Schurig V, Nowotny H-P, Schmalzing D. Gas-chromatographic enantiomer separation of unfunctionalized cycloalkanes on permethyl-β-cyclodextrin. Angew Chem Int Ed Engl 1989;28:736–737.
9. Meierhenrich UJ, Thiemann WH-P, Goesmann F, Roll R, Rosenbauer H. Enantiomer separation of hydrocarbons in preparation for ROSETTA's "Chirality-Experiment". Chirality 2001;13:454–457.
10. Meierhenrich UJ, Nguyen M-J, Barbier B, Brack A, Thiemann WH-P. Gas chromatographic separation of saturated aliphatic hydrocarbon enantiomers on permethylated β-cyclodextrin. Chirality 2003; 5:S13–S16.
11. Fischer P, Aichholz R, Bölz U, Juza M, Krimmer S. Polysiloxane-anchored permethylated β-cyclodextrin—a chiral stationary phase with broad applications for gas chromatographic enantiomer separation. Angew Chem Int Ed 1990;29:427–429.
12. König WA. Forum: collection of enantiomer separation factors obtained by capillary gas chromatography on chiral stationary phases. J High Resolut Chromatogr 1993;16:312–323.
13. König WA, Icheln D, Runge T, Pforr I, Krebs A. Cyclodextrins as chiral stationary phases in capillary gas chromatography. Part VII: cyclodextrins with an inverse substitution pattern - synthesis and enantioselectivity. J High Resolut Chromatogr 1990; 13:702–707.
14. Kubinec R, Soják L, Mračnová R, Kudláčová G, Bohac A. The separation of stereoisomers using recycle capillary gas chromatography. Enantiomer 1999;4:345–350.
15. Eyres GT, Urban S, Morrison PD, Dufour JP, Marriott PJ. Method for small-molecule discovery based on microscale-preparative multidimensional gas chromatography isolation with nuclear magnetic resonance spectroscopy. Anal Chem 2008;80:6293–6299.
16. Grynbaum MD, Kreidler D, Rehbein J, Purea A, Schuler P, Schaal W, Czesla H, Webb A, Schurig V, Albert K. Hyphenation of gas chromatography to microcoil 1H nuclear magnetic resonance spectroscopy. Anal Chem 2007;79:2708–2713.
17. Kühnle M, Kreidler D, Holtin K, Czesla H, Schuler P, Schaal W, Schurig V, Albert K. Online coupling of gas chromatography to nuclear magnetic resonance spectroscopy: Method for the analysis of volatile stereoisomers. Anal Chem 2008;80:5481–5486.
18. Buddrus J, Herzog H. Coupling of Chromatography and NMR 3. Study of flowing chromatographic fractions by proton magnetic resonance. Org Magn Reson 1981;15:211–213.
19. Herzog H, Buddrus J. Coupling of chromatography and NMR part 5: analysis of high-boiling gas-chromatographic fractions by on-line nuclear magnetic resonance. Chromatographia 1984;18:31–33.
20. Wu N, Peck TL, Webb AG, Magin RL, Sweedler JV. ^{1}H NMR spectroscopy on the nanoliter scale for static and on-line measurements. Anal Chem 1996;66:3849–3857.
21. Behnia B, Webb AG. Limited-sample NMR using solenoidal microcoils, perfluorocarbon plugs, and capillary spinning. Anal Chem 1998;70:5326–5331.
22. Wilson SR, Malerod H, Petersen D, Simic N, Bobu MM, Rise F, Lundanes E, Greibrokk T. Controlling LC-SPE-NMR systems. J Sep Sci 2006;29:582–589.
23. Wilson SR, Malerod H, Petersen D, Rise F, Lundanes E, Greibrokk T. An alternative multiple-trapping LC-SPE-NMR system. J Sep Sci 2007;30:322–328.
24. Bieri S, Varesio E, Veuthey JL, Munoz O, Tseng LH, Braumann U, Spraul M, Christen P. Identification of isomeric tropane alkaloids from Schizanthus grahamii by HPLC-NMR with loop storage and HPLC-UV-MS/SPE-NMR using a cryogenic flow probe. Phytochem Anal 2006;17:78–86.
25. Jaroszewski JW. Hyphenated NMR methods in natural products research, Part 2: HPLC-SPE-NMR and other new trends in NMR hyphenation. Planta Med 2005;71:795–802.
26. Schurig V. Peak coalescence phenomena in enantioselective chromatography. Chirality 1998;10:140–146.