Original

Evaluation of Accuracy for the Quantitative Analysis Using Nuclear Magnetic Resonance as a Detector of HPLC

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Abstract

Continuous−flow LC−NMR technique was applied for the purity assessment of *o*−xylene. Two consecutive LC−NMR runs of a sample and a standard were measured. The purity was calculated by the ratio of the set of data. With such an approach, sources of NMR problems were overcome because identical condition can be obtained for both the standard and the sample during NMR measurements. As a result, significant reduction of dispersion and considerable improvement of accuracy in the results were obtained compared to the previous work reported. Comparison of the result of purity assessment obtained with LC−NMR to GC−FID and differential scanning calorimeter (DSC) showed excellent agreement. With such an NMR experimental condition, on−flow NMR can provide accurate and precise results as a detector of HPLC.

Key words:

Introduction

LC−NMR has been extensively used for determination and elucidation of chemical structures [1]. In such applications, NMR often suffers poor sensitivity of signal detection. Therefore, stop−flow LC−NMR method, in which flow of LC is stopped when an analyte enters NMR detector, is widely used in order to obtain spectra with sufficient signal to noise ratio to analyze. NMR has been also known that the area integration of the peaks is proportional to the number of nuclei; thus it can be applied to the quantitative analysis. However, accuracy and precision of quantitation using NMR are often poor because quantitative NMR requires its special parameter settings [2], which are quite different from those for structural determinations.

Application of LC−NMR to quantitative analysis was reported with continuous−flow method [3], which used NMR as a detector of HPLC much like the conventional detectors such as UV. One of the requirements in quantitative NMR is a long relaxation delay; at the mean time, relaxation delay in continuous−flow LC−NMR is limited by flow rate. This contradicted problem must be overcome for obtaining the accurate continuous−flow LC−NMR measurements. Previous reports showed accuracy of the result was about 10%, precision was indicated worse.

Here we aimed to obtain better accuracy of *o*−xylene purity assessments using NMR as a detector of HPLC. Our approach was following; two consecutive continuous−flow LC−NMR runs were measured; the *o*−xylene sample was measured during the first run, and the standard sample was measured during the second run. The ratio of NMR area integrations obtained by these two runs leaded the purities. In such a condition, we could maintain identical detection conditions in NMR measurements between the sample and the standard. The NMR results were compared with results obtained with UV detector. Furthermore, the results obtained with LC−NMR, GC−FID, and DSC were also compared.

Experimental

Materials

Sample: *o*−xylene (Wako Pure Chemical Industries, Ltd)

Standard sample: *o*−xylene (National Metrology Institute of Japan, AIST, (NMIJ CRM 4011 a), purity (99.940 ± 0.003) wt/wt %)

HPLC eluents: D_2O and CD_3CN were obtained from Acros Organ-

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ics.

HPLC

HPLC was performed on Agilent 1100 HPLC system, with a pump, an auto sampler, a column oven, and a diode array detector (DAD) operating at 290 nm. The columns used in this study were two L−column ODS (250 x 4.6 mm i.d., 5 μm packing, Chemicals Evaluation and Research Institute, Japan) connected in series. The HPLC conditions were as follows; the flow rate was maintained at 1 mL/min, the elution mode was linear gradient started with 40 vol/vol % CD_3CN in D_2O to 70 vol/vol % CD_3CN in D_2O during 50 minutes, and the sample injection was 3 μL, where neat samples were injected. The sample temperature in the sample tray of the auto sampler was kept at 5℃, and the column temperature was controlled at 40℃. The sample was rather overloaded for this column because NMR is not so sensitive instrument compared with other chromatographic detectors such as UV and FID. By this chromatographic condition, base line separation was obtained between *o*−xylene and the other xylene isomers.

NMR

NMR was on−line connected after the DAD via PEEK tube. Thus, this LC−NMR system consisted of HPLC system including such as injector, columns, and detector, and NMR connected in series. All NMR spectra were acquired with Varian ^{UNITY} INOVA 600 MHz instrument with a Varian Interchangeable Flow Cell Micro Flow indirect detection probe ^1H {$^{13}C/^{15}N$} with z−axis gradient. The following parameters were used for the NMR measurements; an 18 315.0 Hz spectral window, an 18 000 Hz filter bandwidth, a 2.2 μs (30 °) ^1H pulse width. Acquisition time and relaxation delay were maintained at 1.79 s and 0.01 s, respectively; in other words, every 1.8 s, NMR spectrum was acquired, which was not an ordinary parameter setting for the quantitative NMR measurement. Temperature at the NMR detector was controlled at 25℃.

LC−NMR

NMR data acquisition was started 37 minutes after the sample was injected into the column; two transients were averaged for each of 256 increments, which covered entire peak area of *o*−xylene. After correcting the pseudo two−dimensional LC−NMR data, aromatic ^1H peaks were integrated along time dimension for the determinations. Alternative LC−NMR runs were measured for the standard and the sample.

Results and discussion

Figure 1 shows UV chromatogram of the sample. Trace amount of impurities were found and were perfectly separated from the main peak. In Figure 2, LC−NMR spectrum of the sample is shown, which displays only aromatic resonance in ^1H NMR dimension. Because of its high selectivity of NMR and complete separation condition of HPLC, no peak from impurity was found. Two consecutive runs were performed between the standard and the sample. From the area integrations of the two runs, the results were calculated by the following equation,

$$P_x = \frac{I_x}{I_{st}} P_{st} \qquad (1)$$

where P stands for the purity, subscripts x and st are the samples and the standard, respectively, I is the area integration of NMR peak or UV chromatogram. Table 1 summarizes the purities and relative standard deviations (RSD) obtained with UV and NMR. Good agreements of results between NMR and UV detectors were

Table 1. Comparison of results and RSD obtained from NMR and UV detectors.

Method	Purity (%)	RSD (%)
NMR	99.7	0.73
UV	98.7	0.34

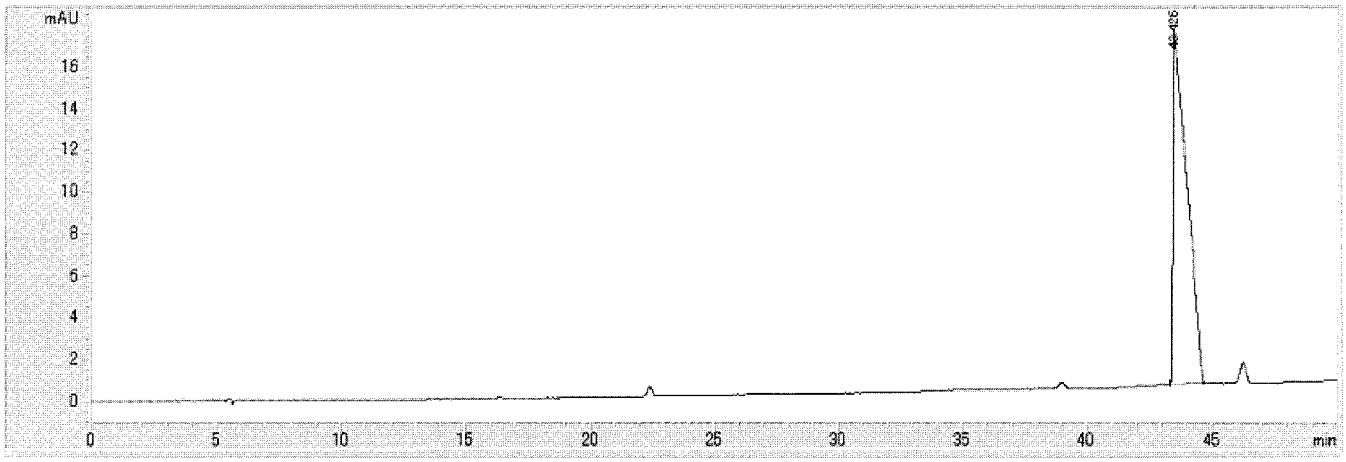

Figure 1. Chromatogram of the commercially available *o*−xylene sample. The peak eluted at 43 minutes is the peak from *o*−xylene.

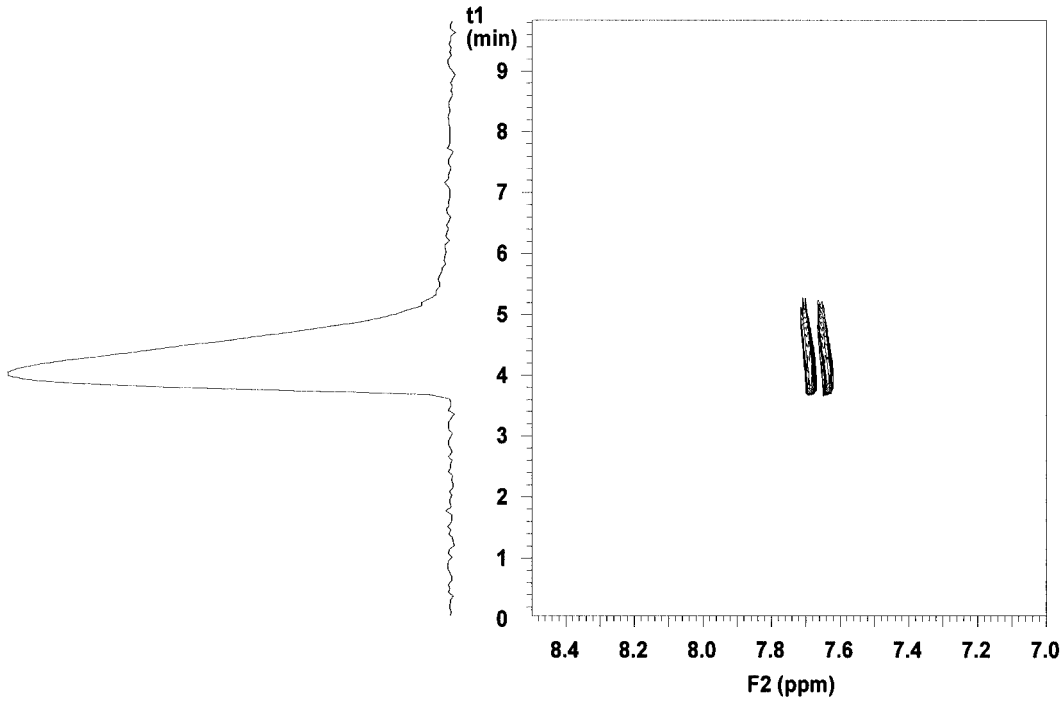

Figure 2. LC−NMR spectrum of the commercially available *o*−xylene sample. Horizontal axis corresponds chemical shift of NMR and vertical axis is a retention time of HPLC. The time at zero corresponded 37 minutes after the auto sampler of HPLC injected the sample. Chromatogram plotted on the left side of the LC−NMR spectrum was obtained by summing the NMR signals between 7.5 and 7.9 ppm.

obtained with this method.

Previously, three methods were reported. The first one was an internal standard was dissolved in eluent, which might cause problems of optimizing a separation condition. The second method was that an internal standard was added to the sample. In either case, structures could not be matched to the analyte. The third way was to inject a standard at slight different time. However, this was impossible when gradient method was used. When structures of the sample and the standard were different, it caused the problems in accuracy of NMR measurements.

Since accurate quantitation with NMR requires a long relaxation delay, for example 60 s. Such long delay cannot be set for continuous−flow NMR parameter because time resolution of, such as, 60 s is equivalent to or longer than peak bandwidth of HPLC peaks. When short relaxation delay is used, samples start to give inaccurate NMR response due to saturation of the sample molecules. Each kind of molecule has different relaxation properties; put it differently, the rate of the saturation is different. The comparison between different molecules in such a condition provided accuracy in the results obtained from NMR. On the other hand, we used the same material as a standard; the relaxation properties were identical when NMR signals were acquired. Consequently, we can overcome the problems of the saturation; the results obtained by UV and LC−NMR detectors agreed within 1%, which was a significant improvement compared to the previous works. The RSD of the NMR results was also improved significantly, which suggested perfect control of relaxation properties. The controlled properties leaded the accurate results.

We also compared the purity of a sample, whose purity was prepared around 98%, determined by LC−NMR, GC−FID and DSC methods. The deviation of the results obtained from these three methods was less than 0.1% that strongly proved the accuracy of this continuous−flow LC−NMR method.

Conclusions

We demonstrated the feasibility of continuous−flow LC−NMR as a detector of. The problems caused by slow process of NMR such as relaxation process was overcome by the use of the identical standard. The quantitative analysis of continuous−flow LC−NMR using our parameter setting had comparable abilities in both the result of the purity and the repeatability to those by UV detector. When NMR was used as the detector for LC, no sample preparation process was necessary for the purity assessment, but neat sample was directly injected into HPLC. Since LC−NMR spectrum provides chemical shift dispersion along with the retention time of HPLC, the technique can overcome problems of co−

elution of the samples. The result suggests the possibility of continuous−flow LC−NMR as a tool of quantitative analysis, where stop−flow LC−NMR method, which is used in applications of structural determination, is not feasible because sample detection volume of NMR is not as large as the volume of sample solutions. In such a condition, repeatability and reproducibility cannot be obtained. In the metrology in chemistry, ^{1}H NMR is consider to be a primary method [4], whose result of measurements is traceable to SI and does not need referring a same amount of standard. The result strongly supports the possibility of not only the ordinary NMR, but also LC−NMR to be the primary method of measurements.

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