



## Quantitative Analysis of Chloride by Heteronuclear Electronic Reference NMR Method

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**Abstract** : A new quantitative analysis of chloride by the HERETIC NMR method which does not need internal or external references was described. The results showed that the use of HERETIC peak corresponding 500  $\mu\text{g/mL}$  of chloride calibration showed less than 4 % standard deviations from 50 to 5000  $\mu\text{g/mL}$  range of chloride concentrations.

Keywords : Quantitative Chloride Analysis, Heteronuclear Electronic reference, Quantitative NMR Analysis

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the most important instrumental methods to give the structures and dynamics of the molecules. However, among the many analytical instruments, NMR was distinctly unpopular for the quantitative analysis until recently because of the many inferior intrinsic aspects, particularly its sensitivity. In spite of those defects, NMR has been continuously used for the quantitative analysis since the first appearance of NMR instruments because the direct proportionality of the peak intensity to the concentration of the individual compound is the most powerful aspect which could not be found in any other instruments.<sup>1</sup> Recently, NMR is increasingly used in quantitative applications with sensitivities into the parts per billion range with the introduction of high field NMR instruments. These applications

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include binding studies for drug screening, metabolite identification and trace level analysis of impurities such as residual solvents. Although the significant development of NMR technology enables the NMR method to be used more easily for the quantitative analysis, its applications are generally limited to the use of hydrogen nucleus with several experimental difficulties.<sup>2</sup>

In continuing study of heteronuclear NMR application in quantitative analysis, we previously reported the analysis of chloride in solution by using external reference method<sup>3</sup> because chloride is one of the most frequently analyzed elements in various field of science as a unique versatile probe.<sup>4,5</sup> The content of chloride could have even been a determinant for the diagnosis of human cystic fibrosis.<sup>6</sup> Although the quantitative analysis of chloride can be done by many different methods such as gravimetric, coulometric, titrimetric, colorimetric, ion chromatographic, and etc,<sup>7,8</sup> these methods, however, could have several matrix effect which disturb to get correct analysis results.<sup>9</sup> On the other hand, the external reference NMR method showed that NMR method was independent of the matrix effect and relatively simple and easy to implement in comparison with the other analytical methods. Because of the natural abundance (chlorine-35 = 75.5 %) and short relaxation time caused by quadrupole, Chlorine-35 is especially a very amenable nucleus to measure NMR signal.<sup>10</sup> With this approach, we could easily analyze the chloride in several different matrices in the range of 20 to 5000  $\mu\text{g/mL}$ . However, the external reference NMR method still has several intrinsic drawbacks to implement such as choosing the appropriate chemical shift reagent to separate the resonances of sample and reference.<sup>3,11</sup> and the cumbersome exploitation of an external reference.

We recently reported that the proton coil can be used as a broad band antenna to detect the heteronuclear pseudo-FID generated by multiplication of an exponential function and heteronuclear frequency and this easily extends the method to the measurement of heteronuclear species concentrations without the difficulties associated with chemical references.<sup>12</sup> This HERETIC signal could have been observed even at the chlorine-35 resonance position which was about 10 times lower than the tuned frequency.

This paper will describe a new quantitative analysis of chloride by the HERETIC NMR method which does not need internal or external references including the chemical shift reagent to separate the resonances of sample and reference.

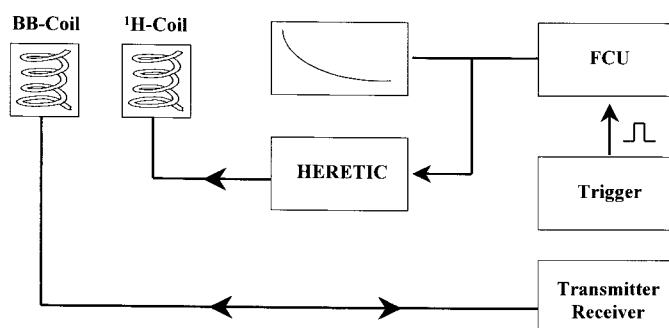


Fig. 1. Block diagram for the HERETIC Method.

The reference signal obtained by the Heteronuclear Electronic REference To access In vivo Concentration (HERETIC) method is a pseudo-FID produced on a proton coil of a broad band probe by multiplication of an exponential wave function as a low frequency element and heteronuclear frequency generated from frequency control unit (FCU) in Bruker AVANCE-700 spectrometer. The high frequency component in reference signal was provided during the acquisition time of observing channel by the second channel of the spectrometer using “eretic” pulse program of Bruker. It was derived from the FCU and modulated with low frequency element as a shape pulse which was sent directly to the proton coil of broad band probe without amplification. Therefore, the phase of reference signal is independent of the transmitter pulse and its magnitude and frequency can be freely determined as decoupler parameters in pulse program by the operator. The broad band channel was used for transmitting corresponding rf hard pulses and receiving the real and pseudo-FID signal (Fig. 1).

The chlorine-35 was observed on Bruker AVANCE-700 spectrometer at 68.61 MHz. HERETIC pseudo-FID signals defined by frequency and amplitude modulated by several different powers were acquired with the real FID signals derived from the real samples contained in 5 mm NMR tube and showed in Fig. 2. The power of the shape pulse was attenuated from 4 dB to 28 dB by 4 dB units and the frequency of HERETIC signal was placed at  $-50$  ppm. The signal intensities obtained from the 200  $\mu\text{g/mL}$  of chloride

solution are constant while the intensities of HERETIC signal vary depending on the power of shape pulse.

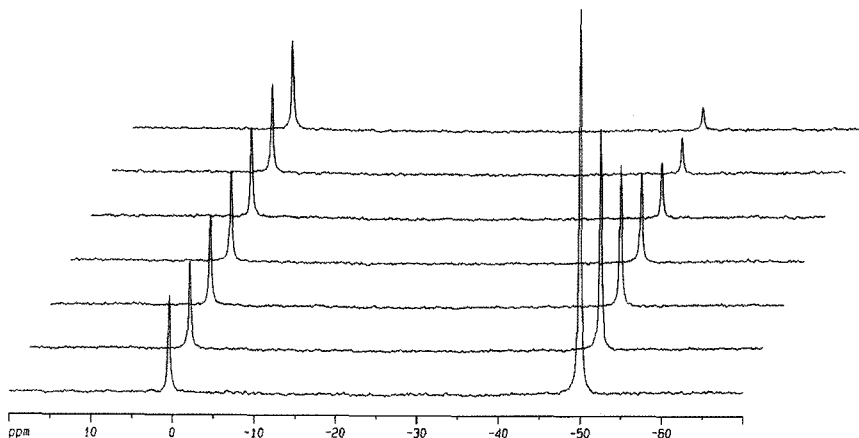


Fig. 2. HERETIC signals at  $-50$  ppm modified by the power of the shape pulse relative to the  $200 \mu\text{g/mL}$  chloride solution.

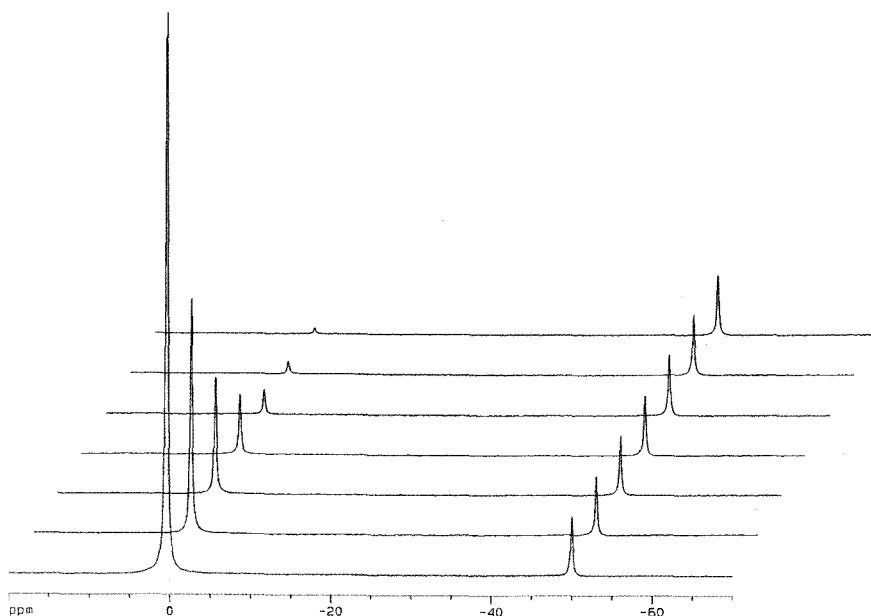


Fig. 3. Chlorine-35 NMR spectra ( $5000, 2000, 1000, 500, 200, 100, 50 \mu\text{g/mL}$ ) with the HERETIC signals at  $-50$  ppm calibrated as  $500 \mu\text{g/mL}$  sodium.

To prove the applicability of this method, the Fourier transformed reference signal calibrated against the known standard solution was used for the quantitative analysis of chloride in solution which was one of the most intensively studied method.<sup>4,5,6</sup> Using the power of shape pulse and spectrum width, the intensity and line width (*ca.* 20 Hz) of the HERETIC signal was adjusted to have similar shape as that of standard solution to reduce the error of integration. Fig. 3 shows representative chlorine-35 NMR spectra obtained from the different concentrations of known samples, 50, 100, 200, 500, 1000, 2000, and 5000  $\mu\text{g/mL}$  chloride solution, respectively, each compared with the HERETIC signal calibrated as 500  $\mu\text{g/mL}$  of chloride. The spectra were collected with fast pulsing due to the quadrupole relaxation of chloride and the HERETIC signal which was free from the relaxation delay: 0.2 s relaxation delay, 0.3 s acquisition time, and 8 K data points over a 13370 Hz spectral width using  $90^\circ$  pulse. 17 minutes of acquisition (NS=2K) were enough to give reasonable signal-to-noise ratio even for 50  $\mu\text{g/mL}$  of sodium solution. An exponential multiplication (LB=10) was applied to the FID prior to FT and 5<sup>th</sup> order polynomial baseline corrections were employed.

The area ratio of HERETIC and sample peaks obtained from the standard samples was used to measure the concentrations of different sample directly because of good stability of HERETIC peak. The area deviation of HERETIC peak relative to the known chloride concentration (200  $\mu\text{g/mL}$ ) was less than 1 % for 1 week with the same probe tuning and power level.

The use of HERETIC peak corresponding 500  $\mu\text{g/mL}$  of chloride calibration showed less than 4 % standard deviations over the entire range of concentrations determined in this experiment. With the exception of the 20  $\mu\text{g/mL}$  of sodium solution (> 5 %), the application of a 200  $\mu\text{g/mL}$  of chloride calibration gave comparable results as that of 500  $\mu\text{g/mL}$  of chloride calibration.

The chemical reference (internal and external) methods for the measurement of chloride concentration as well as the other heteronuclei has the intrinsic drawbacks generally associated with external references including the need for chemical shift reagent to separate the resonances of sample and reference.<sup>3,11</sup> However, the HERETIC method used here could get rid of not only the difficulty of chemical shift reagent but also the

cumbersome exploitation of external reference to measure the concentration of chloride in solution..

In conclusion, this HERETIC method could overcome all the problems produced by chemical references and could be the other alternative standard method with several merits to measure chloride concentrations.

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### **REFERENCES**

1. J. N. Shoolery, *Prog. NMR Spectroscopy* **11**, 79 (1977) and references cited therein.
2. J. Mendham, R. C. Denney, J. D. Barnes, M. J. K. Thomas, In *Vogel's Quantitative Chemical Analysis* 6th ed., Prentice Hall: England, 2000 Chap. 14 and references cited therein.
3. H.-S. Lim, Lee, S.-G. *Bull. Korean Chem. Soc.* **27**, 972 (2006).
4. M. Biesaga, N. Schmidt, A. Seubert, *J. Chromatogr. A* **1026**, 195 (2004).
5. L. E. Vanatta, D. E. Coleman, A. Woodruff, *J. Chromatogr. A* **997**, 195 (2003) and references cited therein.
6. M. Hukelmann, O. Oster, *Clinica Chimica Acta* **319**, 75 (2002) and references cited therein.
7. W. J. Williams, *Handbook of Anion Determination* Butterworths: London, 1979.
8. D. F. Boltz, J. A. Howell, *Chemical Analysis* 2nd ed., John Wiley and Sons: USA, 1978; Vol. 8.
9. Z. Sulcek, P. Povondra, *Methods of Decomposition in Inorganic Analysis* CRC Press: FL. 1989.
10. J. W. Akitt, In *Multinuclear NMR* J. Mason, Ed.; Plenum Press: New York, 1987; Chap.7.

11. H.-S. Lim, G.-C. Han, S.-G. Lee, *Bull. Korean Chem. Soc.* **23**, 1507 (2002) and references cited therein.
12. S.-G. Lee, *Bull. Korean Chem. Soc.* **2007**, in press.