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# Determination of Absolute Content of Cyetpyrafen by qNMR Analysis

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## Abstract

This paper established a rapid, simple method for determination of absolute content of cyetpyrafen by qNMR. $^1$H NMR spectra was acquired with deuterodimethyl sulfoxide (DMSO-D6) as the solvent and hydroquinone as internal reference standard under the conditions of temperature 25℃, pulse angle 90°, pulses width 8.0 μs, relaxation delay 30 s, and scans 8. The proton peaks of cyetpyrafen (δ=6.35) and hydroquinone (δ=6.55) were taken as quantitative peaks. The peak area ratio $y$ ($A_s/A_r$) and concentration ratio $x$ ($C_s/C_r$) were linearly regressed, and the correlation coefficient was 0.9999. The *RSD* value of repeatability test was 0.80%, and the *RSD* value of stability test was 0.54%. The content of cyetpyrafen was determined as 99.8%. The result shows that qNMR can be used for quantitative determination of cyetpyrafen without reference standard, which is rapid, accurate and simple.

## Keywords

Cyetpyrafen, qNMR, Internal reference standard, Absolute Content

## 1. Introduction

Chemical pesticides have an irreplaceable role in comprehensive prevention and control on crop pests and diseases [1]. Cyetpyrafen (SYP 9625) is a potent acaricide discovered by Shenyang Sinochem Agrochemicals R&D Co., which was discovered through structural modification with pyrazolyl acrylonitrile derivatives as a lead compound [2]. Cyetpyrafen belonging to mitochondrial electron transport inhibitors of complex II (METI II) has been widely applied to manage pest mites in China [3]. So far, the content of cyetpyrafen is determined by high performance liquid chromatography (HPLC) [4]. However, quantitative nuclear magnetic resonance (qNMR) was developed to determine content in drug, metabolite, etc., has been widely used in various fields, including chemistry, biology, food, agriculture and medicine. Although NMR is less sensitive and specific than HPLC, qNMR method has some advantages over chromatographic methods [5, 6], it could be qualitative and quantitative in one analysis. The qNMR technology is a new and important quantitative analysis technique for the purity determination of standard substances because of its characteristics of simple pretreatment, fast determination and non-destruction of samples. This technology has been adopted as the standard method by ChP [7].

In this paper, a method for determination the absolute content of cyetpyrafen has been established by using qNMR spectroscopy method, which could determine the absolute content without reference standard substance, being of great significance for quality control of cyetpyrafen and its formulations.

## 2. Materials and Methods

### 2.1 Instruments

600 MHz NMR spectrometer (Jeol JNM-ECZ600R) and electronic balance (Mettler XP6, Max = 6.1 g, d =1 μg).

## 2.2 Reagents

Deuterodimethyl sulfoxide (DMSO-D6, D 99.9% and 0.03% TMS) was purchased from Sigma-Aldrich, hydroquinone (GC, 99.5%) was purchased from Aladdin, and cyetpyrafen standard was provided by Shenyang Sinochem Agrochemicals R & D Co. The structural formula of cyetpyrafen is shown in Figure 1.

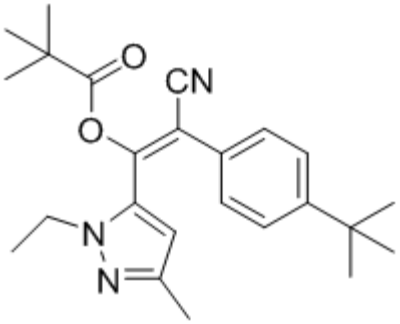


**Figure 1. Structural formula of cyetpyrafen.**

## 2.3 Experimental conditions

In this procedure, the NMR spectra was collected under following conditions: temperature 25℃; pulse angle 90°, pulse width 8.0 μs; relaxation delay 30 s; scans 8.

## 2.4 Preparation of samples

### 2.4.1 Internal reference standard solution

8 mg (± 0.01mg) hydroquinone was accurately weighed and dissolved in 10mL (±0.01mL) DMSO-D6, preparing to 0.7 mg/mL solution.

### 2.4.2 Sample solution

In order to prepare sample solution, 6 mg cyetpyrafen (± 0.01 mg) was accurately weighed and put into a 5 mm NMR tube, and dissolved in 0.5 mL internal reference standard solution, mixed the solution until obtained a clear solution.

## 2.5 Determination method

NMR spectra were collected under acquisition conditions stipulated in "2.3". The quantitative peaks of cyetpyrafen and hydroquinone were integrated respectively, and the content of cyetpyrafen in the sample was calculated by the following formula:

$$W_s(\%) = \frac{(\frac{A_S}{N_s}) \times M_S \times C_r}{(\frac{A_r}{N_r}) \times M_r \times C_s} \times W_r$$

Here, $A_s$ is the peak area of cyetpyrafen; $A_r$ is the peak area of hydroquinone; $N_s$ is the proton number of the quantitative peak of cyetpyrafen; $N_r$ is the proton number of the quantitative peak of hydroquinone; $C_s$ is the concentration of cyetpyrafen; $C_r$ is the concentration of hydroquinone; $M_s$ is the molecular mass of hydroquinone; $W_r$ is the content of hydroquinone.

# 3. Results and Discussion

## 3.1 Selection of internal reference standard and quantitative characteristic peak

Cyetpyrafen were dissolved in DMSO-D6 and 1H NMR spectra were collected. Obtained spectra is given in Figure 2. The peak attribution of cyetpyrafen was Proton-NMR (600 MHz, DMSO-D6) δ 7.43 (d, J = 8.3 Hz, 2n), 7.09 (d, J = 8.3 Hz, 2n), 6.35 (s, 1n), 3.50 (q, J = 7.1 Hz, 2n), 2.16 (s, 3n), 1.26 (d, J = 21.3 Hz, 18n), 0.83 (t, J = 7.2 Hz, 3n). The results showed that there were isolated unimodal peaks when δ = 6.35, and there was no interference with peaks when δ = 6.35, so the proton with δ = 6.35 was selected as the characteristic peak for quantitative investigation.

A good internal reference standard should meet the following conditions: a. do not react with any component in the sample; b. preferably show a single peak; c. should be dissolved in a deuterated reagent. In this paper, maleic acid, dimethyl terephthalate and hydroquinone were tried to be the solvent. The solubility and characteristic peaks of the above internal reference standards in DMSO-D6 were investigated respectively. The results are shown in Table 1. The three internal reference standards are all soluble in DMSO-D6, but the chemical shift of the characteristic peak of maleic acid is too close to those of cyetpyrafen, dimethyl terephthalate has two sets of signals, hydroquinone has only one set of

signals, and it was well separated from the cyetpyrafen peak, therefore hydroquinone was finally chosen to be the internal reference standard. The proton peak of hydroquinone with δ = 6.55 was chosen as the characteristic peak for quantitative analysis. The $^{1}$H NMR spectra of sample solution is given in Figure 3.

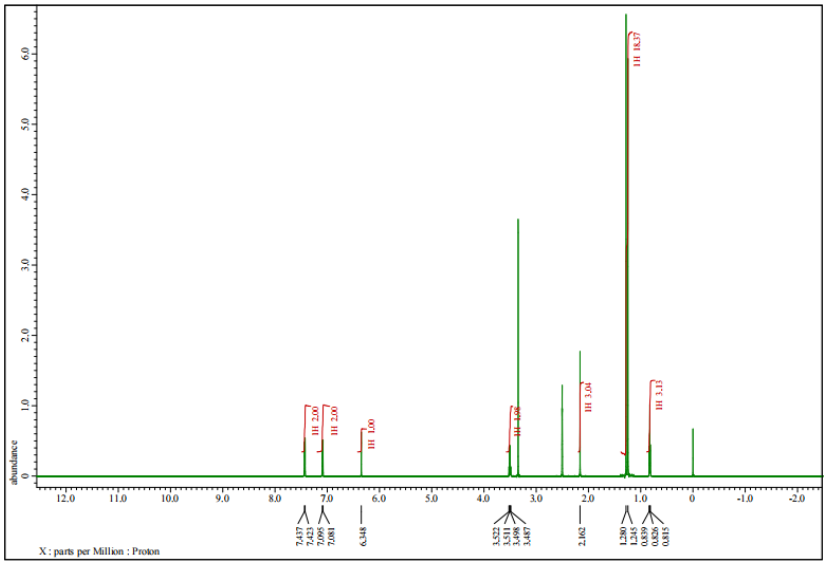


**Figure 2. $^{1}$H NMR spectra of cyetpyrafen.**

**Table 1. Internal Reference Standard Study Result**

| internal reference standard | solubility(DMSO-D6) | chemical Shifts |
|---|---|---|
| maleic acid | √ | 6.32 |
| dimethyl terephthalate | √ | 8.11; 3.94 |
| hydroquinone | √ | 6.55 |

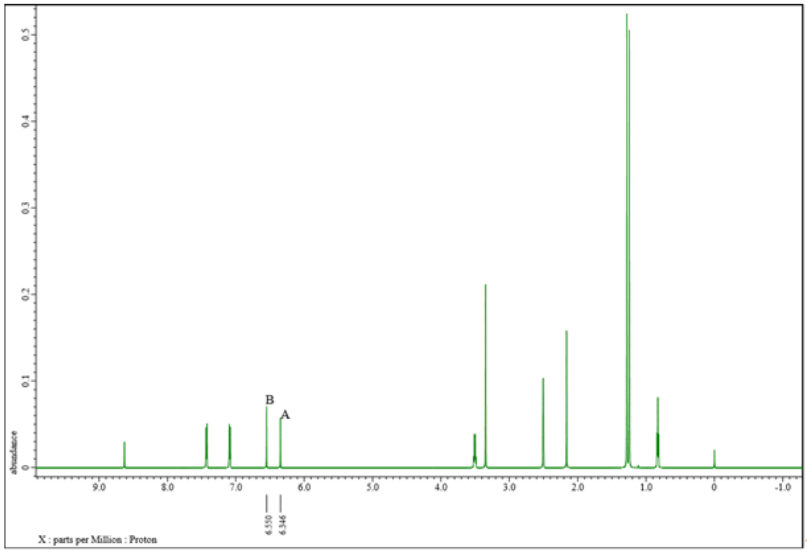


**Figure 3. $^{1}$H NMR spectra of test samples.**

Note: A. Quantitative peak of cyetpyrafen; B. Quantitative peak of hydroquinone.

## 3.2 Limit of quantification (LOQ)

The limit of quantification (LOQ) for cyetpyrafen was determined at a signal-to-noise ratio of 10:1, by using a series of dilute solutions, which had known concentrations of cyetpyrafen. LOQ was obtained at 11.91 μg/mL.

### 3.3 Linear relation

In order to perform the linearity study of cyetpyrafen, five different concentration solutions were prepared. Firstly 4, 6, 8, 10 and 12 mg of cyetpyrafen were weighed respectively and were dissolved in 0.5 mL internal reference standard solution, then linearity study sample solution were obtained. The $^1$H NMR spectra were collected under the NMR acquisition conditions described in "2.3", and the characteristic peaks of cyetpyrafen and hydroquinone were integrated, respectively.

Least squares linear regression was performed using the peak area ratio *y* of cyetpyrafen and hydroquinone and the mass ratio *x* of cyetpyrafen and hydroquinone, and the regression equation was $y$=0.0706x-0.0082, with the correlation coefficient $r$=0.9999. The results showed that the content of cyetpyrafen was determined by qNMR in the concentration range of 8 ~24 mg/mL.

### 3.4 Repeatability

In order to perform the repeatability study of cyetpyrafen, 5mg cyetpyrafen (± 0.01 mg) was accurately weighed and put into a 5 mm NMR tube, and dissolved in 0.5 mL internal reference standard solution, and six sample solutions were prepared respectively. The absolute content of cyetpyrafen in six solutions was determined respectively, and the *RSD* value was 0.80%. The results showed that this method had excellent repeatability. The results of repeatability are presented in Table 2.

**Table 2. Repeatability of determination of cyetpyrafen content (n=6)**

| No. | $m_s$ (mg) | $A_s/A_r$ | $W_s$ (%) | *RSD* (%) |
|---|---|---|---|---|
| 1 | 5.407 | 0.97 | 101.2 | 0.80 |
| 2 | 5.456 | 0.96 | 99.3 | |
| 3 | 5.257 | 0.93 | 99.8 | |
| 4 | 5.193 | 0.91 | 98.9 | |
| 5 | 5.034 | 0.89 | 99.8 | |
| 6 | 5.083 | 0.90 | 99.9 | |

### 3.5 Stability of solution

The No. 2 sample in "3.3" was stored at room temperature for 24h, and measured at 0, 4, 8, 12 and 24 h, respectively. The content of cyetpyrafen was calculated, and the *RSD* value was 0.54%. The results showed that the sample was very stable within 24 h after preparation. The results are shown in Table 3.

**Table 3. Stability of cyetpyrafen solution**

| Time(h) | $A_s/A_r$ | $W_s$ (%) | Mean (%) | *RSD* (%) |
|---|---|---|---|---|
| 0 | 0.96 | 99.3 | 99.6 | 0.54 |
| 4 | 0.97 | 100.3 | | |
| 8 | 0.96 | 99.3 | | |
| 12 | 0.96 | 99.3 | | |
| 24 | 0.96 | 99.3 | | |

### 3.6 Accuracy

The accuracy study of the qNMR method was determined by comparing with another method. The content of cyetpyrafen was determined by qNMR method and HPLC method respectively. The content determined by qNMR was 99.8%, compared with the content determined by HPLC (99.6%). There are no significant differences in results, and the relative error with the HPLC content was 0.2%.

## 4. Conclusion

The absolute content of cyetpyrafen sample was determined by qNMR and compared with the HPLC content. The results showed that the method was rapid and accurate, which had good linearity, repeatability, solution stability and accuracy. It could not only be used to determine the absolute content of cyetpyrafen without reference standard, but also

could be used to determine the content of cyetpyrafen and its formulations.

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