Application Data Set from Shimadzu

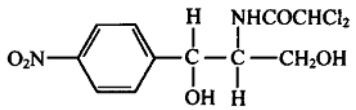
●LCMSMS Analysis LCMSMS-003

Determination of chloromycetin (chloramphenicol) by ultra fast liquid chromatography-triple quadrupole mass spectrometry

Abstract: Chloromycetin (chloramphenicol) is an abroad-spectrum antibiotic which is banned for animal derived food in many countries because of its hematopoietic function inhibiting action. In this paper, a method is proposed for fast and sensitive determination of chloromycetin with Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method demonstrated good linearity for chloromycetin in the concentration range of 0.05-50 μg/L, with a correlation coefficient of calibration curve higher than 0.9999. Results of precision test on 6 successive injections showed that the %RSDs of retention time were 0.14-0.35% and RSDs of peak area ratio were 2.96-4.16%, suggesting that the system was of good precision. Moreover, the method was highly sensitive and achieved an LOQ of 0.005 ng/mL.

Key words: chloromycetin, triple quadrupole mass spectrometry, ultra fast liquid chromatography

Chloromycetin, also called chloramphenicol, is an abroad-spectrum antibiotic which is commonly used for the treatment of bacterial infectious diseases in fishery and poultry husbandry production. Its chemical structural formula is as follows.



Chlorampenicol(CAP)

Because of the hematopoietic function inhibiting action of chloromycetin, its application in food of animal origin is banned in many countries and an MRL of zero is set for chloromycetin in edible tissues of all food animals. It is stipulated

Author: Yu Yanyan, Shanghai Analysis Center. E-mail: sshyyy@shimadzu.com.cn

by the Ministry of Agriculture of China (in No. 227 announcement of the year 2002) that the aforementioned ban was also applied in China and chloromycetin was included in the *List of Food Additives That May Be Illegally Added into Food and Abused (the fifth batch)*.

Therefore, it is absolutely necessary to develop simple, fast, and sensitive analytical methods for chloromycetin. Ultra fast liquid chromatography (UFLC) comprehensively improves separation efficiency, peak capacity and sensitivity with its 1.6 μm sized filler. The UFLC system was used in conjunction with Shimadzu LCMS-8030 triple quadrupole mass spectrometer to develop a fast and sensitive analytical method which was capable of completing analysis of samples within 1.5 min. The proposed method had an LOQ of 0.05 ng/mL for chloromycetin and met the requirements for analysis of banned veterinary drug residues.

1. Experiments

1.1 Apparatus

A combined system of Shimadzu ultra fast liquid chromatograph LC-30A and triple quadrupole mass spectrometer LCMS-8030 was used in the experiment. The detailed configuration included two LC-30AD pumps, DGU-20A_5 online degasser, SIL-30AC autosampler, CTO-30A column oven, CBM-20A communications bus module, LCMS-8030 triple quadrupole mass spectrometer, LabSolutions ver. 5.41 chromatography workstation.

1.2 Analytical conditions

LC conditions
Column: Shim-pack XR-ODS III 2.0 mm I.D.× 50 mmL., 1.6 μm
Mobile phase A: water
Mobile phase B: acetonitrile
Time program

Time	B Conc
0 min	30%
0.35-0.75 min	90%
0.76-1.5 min	30%

Flow rate: 0.4 mL/min
Column temperature: 40 °C
Injection volume: 20 μL

MS condition
Ionization mode: ESI (-)
Ionspray voltage: -3.0 kV
Nebulizing gas: Nitrogen 3.0 L/min
Drying gas: Nitrogen 15 L/min
Collision gas: Argon
DL temperature: 250 °C
Heater block temperature: 400 °C
Mode: multiple reaction monitoring (MRM)
Dwell time: 30 ms
Pause time: 3 ms
MRM parameters: see Table 1

1.3 Preparation of standard solutions

1 mg/mL standard stock solution was prepared using methanol as solvent. The standard stock solution was then diluted with water into a series of standard working solutions at concentrations of 0.05, 0.1, 0.5, 1, 5, 10 and 50 ng/mL, into which deuterated (d5) chloromycetin was added to serve as internal standard. The spiked level was 1 ng/mL.

Table 1 MRM parameters

Name	Precursor Ion	Product Ion	Q1 Pre Bias(V)	CE(V)	Q3 Pre Bias(V)
Chloromycetin	321.20	152.20	12.0	20.0	29.0
D5-chloromycetin (IS)	326.20	157.20	12.0	15.0	29.0

2 Results and Discussion

2.1 Mass spectrum and MRM chromatogram of chloromycetin

Fig. 1 shows the MS/MS spectrum and Fig. 2 and Fig. 3 shows MRM chromatograms of 1 ng/mL chloromycetin and 1 ng/mL deuterated chloromycetin standard working solutions. In this experiment, chloromycetin was quantitatively assayed using the peak area of product ion at *m/z* 152.20 while deuterated chloromycetin was quantitatively assayed using the peak area of product ion at *m/z* 157.20.

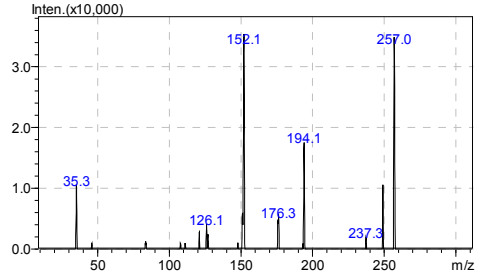


Fig.1 MS/MS spectrum of 1 ng/mL chloromycetin.

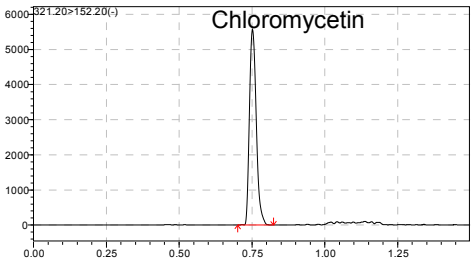


Fig.2 MRM chromatogram of 1 ng/mL chloromycetin (321.20>152.20).

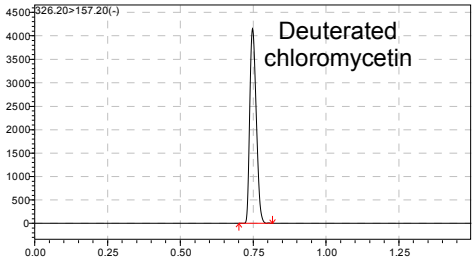


Fig.3 MRM chromatogram of 1 ng/mL deuterated chloromycetin (326.20>157.20).

2.2 Linear range

Multi-standard working solutions at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, and 50 ng/mL were assayed using the analysis conditions under 1.2. Calibration curves were plotted with concentration ratio as abscissa and peak area ratio as ordinate. The calibration curve was of good linearity and had a linear equation of Y=(1.19739)X+(0.195016) and a linear correlation coefficient r=0.99996.

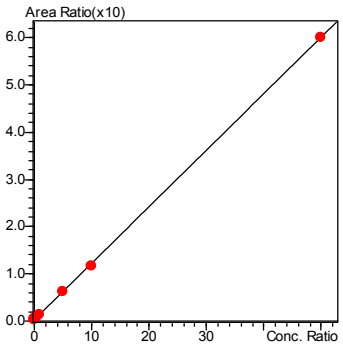


Fig.4 Calibration curve of chloromycetin

2.3 Precision test

Multi-standard working solution was assayed for 6 times in succession to assess the method's precision. The

repeatability results of retention time and peak area are shown in Table 2. The method demonstrated good repeatability.

Table 2 Repeatability of chloromycetin(n=6)

Conc. (ng/mL)	%RSD (RT)	%RSD (Area Ratio)
0.05	0.35%	3.64%
0.1	0.18%	2.96%
0.5	0.33%	3.80%
1	0.23%	3.14%
5	0.20%	4.16%
10	0.29%	3.73%
50	0.14%	4.14%

2.4 Sensitivity test

MRM chromatogram of 0.05 ng/mL chloromycetin standard working solution is shown in Fig.4. Its S/N ratio results are shown in Table 3. The LOQ was calculated to be 0.005 ng/mL.

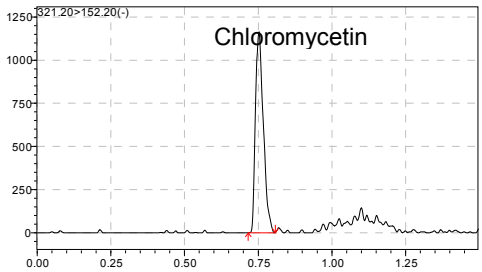


Fig.5 Chromatogram of 0.05 ng/mL chloromycetin(321.20>152.20)

Table 3 S/N ratio of chloromycetin (321.20>152.20, 0.05 ng/mL)

n	S/N ratio
1	90.4
2	79.1
3	137.6
4	141.2
5	89.7
6	101.4
Mean	106.5

3 Conclusion

A method was developed for the assay of chloromycetin with Shimadzu LC-30A ultra fast liquid chromatograph-LCMS-8030 triple quadrupole mass spectrometer. The method was of fast analysis speed and high precision. The %RSDs of retention time and peak area ratio in 6 successive injections were 0.14-0.35% and 2.96-4.16%, respectively. The proposed method had a wide linear range (0.05-50 ng/mL), in which range the correlation coefficient of calibration curve was higher than 0.9999. Moreover, the method was highly sensitive and achieved an LOQ of 0.005 ng/mL. The method demonstrated that Shimadzu LCMS-8030 can be used for highly sensitive quantitative analysis of chloromycetin.