

# GC/MS Analysis of Quinoxaline-2-Carboxylic Acid Residues in Animal Muscle, Liver and Kidney Products

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**Abstract:** The developed GC-MS method provided a high sensitivity and specificity for the analysis of QCA (Quinoxaline-2-Carboxylic Acid) in animal muscle, liver and kidney products. Less interferences and reproducible recoveries were obtained. The minimum detection limit for QCA was 1.0 µg/kg, which was defined on the basis of the current international maximum limit for carbadox and its metabolites residues in animal muscle, liver and kidney products. The recoveries for these samples ranged from 60%~125%. The correlation coefficient was 0.998.

**Key words:** GC-MS; metabolite; QCA; carbadox

## 气质联用法分析动物肌肉、肝脏和肾脏中喹喔啉-2-羧酸的含量

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**摘要:** 本文研究了用气质联用仪检测动物肌肉、肝脏及肾脏中喹喔啉-2-羧酸的含量, 该方法灵敏度较高, 检测低限为 1.0 µg/kg, 干扰较少, 并具有较好的重现性, 可达到 60%~125%, 相关线性系数为 0.998。

**关键词:** 气质联用仪; 代谢产物; 喹喔啉-2-羧酸; 卡巴氧

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### Introduction

Carbadox (methyl-3-(2-quinoxalinylyl-methylene) carbazate- $N^1, N^4$ -dioxide) is an anti-microbial drug used in veterinary practice as an animal feed additive, which has the function of growth promotion, control of dysentery, bacterial enteritis and chemotherapeutic use for swine, but it also has a potential harm to chromosome. Metabolism studies have shown that carbadox could be converted into monooxycarbadox (MCBX) and desoxycarbadox (DCBX) and then Quinoxaline-2-carboxylic acid (QCA) rapidly. Carbadox and one of its intermediate metabolites desoxycarbadox (DCBX) are considered to be carcinogenic and mutagenic. Carbadox is not permitted for use in China. So it is very necessary to develop an analytical method to monitor the presence of carbadox and its toxic metabolite products in animal products. QCA is considered to be the final and the most persistent metabolite and QCA ions are also included in

the monitoring of carbadox.

### Experimental

#### Reagents

Methanol, Metaphosphoric acid, Toluene, Water saturated ethyl acetate, Iso-octane, Trifluoroacetic acid, 0.05 mol/L Sodium hydroxide, 0.1 mol/L disodium hydrogen phosphate buffer solvent (pH 7.0), N-methyl-N-t-butyl dimethylsilyl trifluoroacetamide with 1% T-butyl dimethylchlorosilane (TBDMS), and 2% metaphosphoric acid in 20% methanol: Dissolve 2g of metaphosphoric acid in 80ml water. Add 20ml methanol and mix well by gentle shaking. Store the solution in a glass container at room temperature.

2% trifluoroacetic acid in ethyl acetate: Mix 2ml trifluoroacetic acid with 100ml ethyl acetate. The solution should be freshly prepared before use.

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### Apparatus

Food blender, Analytical balance (capable of measuring to 0.1mg), Vortex mixer, Autopipettes (100 $\mu$ l, 1000 $\mu$ l to 5000 $\mu$ l), Volumetric flasks (10ml, 50ml to 100ml), Nitrogen evaporator, Centrifuge (with temperature control), Polypropylene centrifuge tubes with screw cap (15ml to 50ml) Oasis MAX cartridges (60mg, 3ml), Solid phase extraction vacuum manifold, and Oven (capable of maintaining temperatures at  $(70 \pm 10)^\circ\text{C}$ ).

### GC-MS

Chromatograph equipped with mass selective detector, Agilent 5973 NICI QCA was analyzed by Agilent 6890 gas chromatography and Agilent 5973 mass spectrometry was employed with a Mass-selective detection, Agilent 7683series split/splitless injector, Agilent 7683 series auto sampler, and a HP-5MS fused -silica column (30m  $\times$  0.25mm  $\times$  0.25 $\mu$ m).

The operations condition were:

Injector:  $250^\circ\text{C}$ ; splitless, MS ion source temperature:  $150^\circ\text{C}$ , MS quadrupole temperature:  $150^\circ\text{C}$ , Ionization mode: negative chemical ionization, Carrier gas type: Helium, purity  $>99.999\%$ , Reagent gas type: methane, purity  $>99.995\%$ , acquisition mode: selected-ion monitoring (SIM), voltage: 1600V, and selected ions ( $m/z$ ): 288, 289 and 290. The system was worked at 1.0ml/min with helium. The heater was  $250^\circ\text{C}$  and the aux heater  $280^\circ\text{C}$  respectively. The pressure was 11.7psi. The oven temperature was programmed as follows: the initial temperature was  $80^\circ\text{C}$  and then was increased by  $10^\circ\text{C}/\text{min}$  to  $280^\circ\text{C}$  and held for 3 minutes. The total run time was 23 minutes.

Suggested SIM programme

Retention time (min)	Target analyte	Quantitation ion ( $m/z$ )	Qualifier ions( $m/z$ )	
14.75	Q C A	288	289	290
		100	27.10	11.40

### Preparation

#### Standards preparation

Stock standard solution, quinoxaline-2-carboxylic acid (100mg/L): Weigh accurately about 10mg (to the nearest 0.1mg) of QCA into a 100ml brown volumetric flask. Dissolve the standard and make up to the mark with methanol. Store the solution at  $0^\circ\text{C}$  and avoid direct contact with light.

Working standard solution: Dilute the stock standard solution to the concentration of 1.0, 0.1, 0.05, 0.02, 0.01,

0.005 and 0.001mg/L respectively with methanol. The low working standard solution should be freshly prepared before use.

Preparation of calibration standard solutions:

Add 1ml different concentration of 1.0, 0.1, 0.05, 0.02, 0.01, 0.005 and 0.001mg/L of working standard solution to different tubes respectively. Evaporate the solutions in the tubes carefully to dryness under a gentle stream of nitrogen at  $40^\circ\text{C}$ . Performed the derivatization procedure at  $70^\circ\text{C}$  for 30mins. The final solution in each vial should contain 1, 5, 10, 20, 50, 100 $\mu$ g/L of QCA respectively. Analyzed the standard solutions by GC-NCIMS.

#### Sample preparation

Homogenize the tissue sample by using the food blender. Weigh accurately four equal portions of 2g (to the nearest 0.01g) of homogenized tissue sample into a 50ml centrifuge tube. The first is served as control and the other three portions are spiked with, 1.0, 5.0, 10ng/g (ppb) of QCA respectively.

The homogenised tissues are extracted with 5ml 2% metaphosphoric acid in 20% methanol first. Collect the supernatant after the centrifuge. Add 5ml water saturated ethyl acetate. Combine the upper organic layers with 5ml 0.1mol/L phosphate buffer (pH7.0), and collect the lower aqueous layer after centrifuge.

The aqueous extract is cleaned-up with Oasis MAX SPE cartridge. The column is prewashed with 3ml methanol and then 3ml water. Load the extract into the column. The analytes are washed with 3ml sodium hydroxide solution and eluted with 5ml 2% trifluoroacetic acid in ethyl acetate at a flow rate not exceeding 1ml/min. The final eluent is evaporated to just dryness in a nitrogen evaporator under a gentle stream of nitrogen at  $40^\circ\text{C}$  and reconstituted in 100 $\mu$ l toluene and 100 $\mu$ l MTBSTFA with 1% TBDMCS. After 30minutes derivatization at  $70^\circ\text{C}$ , add 800 $\mu$ l iso-octane to make up to the volume of 1ml, and mix well. The analytes are determined by gas chromatography mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode.

The operation of the blank test is the same as just described in the method of determination, but without addition of sample.

### Results and Discussions

Concentration of quinoxaline-2-carboxylic acid in sample ( $\mu\text{g}/\text{kg}$ ) is:

$$= A \times V/W$$

where  $A$  — concentration of QCA in the sample solution as determined by GC-MS from the calibration curve,  $\mu\text{g/L}$ ;

$V$  — final sample volume, ml;

$W$  — sample weight, gram.

Fig. 1 showed that there was no great interference from 14min to 15min in blank sample, which we could see from Fig. 1 (the chromatograph of the blank sample). QCA in samples was identified by comparison of the retention times and the ion ratios of qualifier ions from the peaks of chromatograph detected. Fig. 2 showed the detection of QCA at a concentration of  $1.0\mu\text{g/kg}$  which could provide the detection limit of  $1.0\mu\text{g/kg}$ . Fig. 3 showed the characteristic mass spectrum of QCA and we could easily verify QCA by the characteristic ions (288, 289 and 290).

According to the recovery experimental data, the recovery

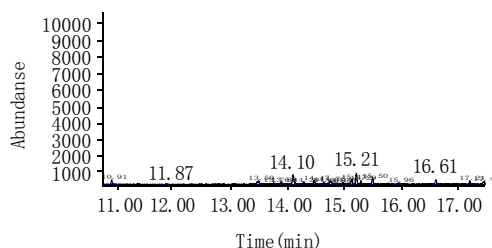


Fig.1. The chromatograph of blank

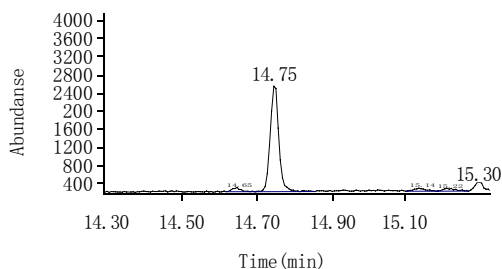


Fig.2 The chromatograph of QCA at 1.0ppb

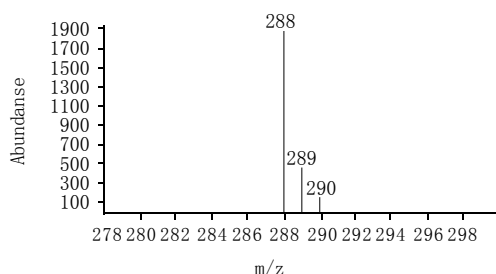


Fig.3 The ion chromatograph of QCA

ery was 85.5% at the fortifying concentration of  $1.0\mu\text{g/L}$ , 98% at  $5.0\mu\text{g/L}$ . The recoveries for these samples ranged from 60%~125%. The chromatographs of recovery experiments

were shown below:

Calibration curves: The area ratios (peak area of QCA)

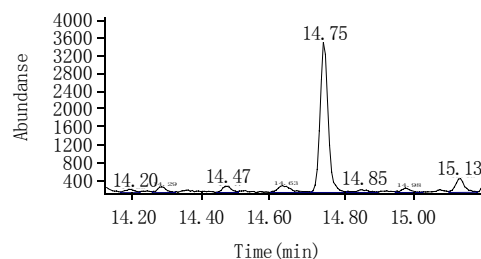


Fig.4 Sample spiked with 1.0ppb

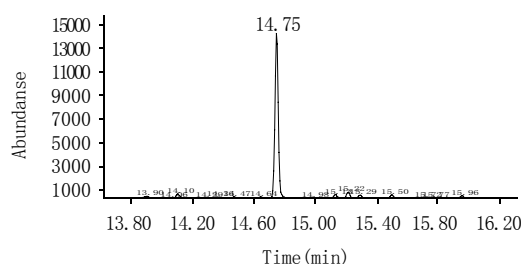


Fig.5 Sample spiked with 5.0ppb

were plotted against concentration ratios (concentration of QCA). The slope, y-intercept and the square of correlation coefficient were obtained from the calibration curves. A five point calibration graph were established by analysis of five calibration standard solutions. Fig. 5 showed that the standard caculation curve and the linearity were good and the correlation coefficient was 0.998.

After precision assays, we could get the RSD of 9.6 at

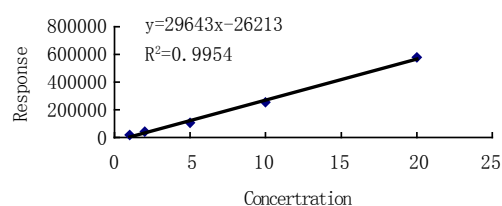


Fig.6 Calibration curve

$1.0\mu\text{g/kg}$  and 5.8 at  $\mu\text{g/kg}$  respectively. In conclusion, this method was verified to be applicable to the determination of QCA in animal muscle, liver and kidney products.

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# 蒸发光散射检测器在番茄红素反相 HPLC 定量分析中的应用

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**摘 要:** 在本项研究中, 应用反相 C<sub>18</sub> 高压液相色谱柱对番茄果实中的番茄红素(Lycopene)进行了分离。色谱条件为: C<sub>18</sub> 固定相: DIAMONSIL™ 柱; 流动相 A: 乙腈: 水 = 9:1; 流动相 B: 乙酸乙酯; 线性梯度洗脱: 在前 15min 内, B 由 0 增为 100%, 随后, B 保持 100%; 流速为 1.0ml/min, 紫外—可见光检测器波长范围: 260~600nm; 监测波长: 475nm; 进样量为 20μl; 柱温: 室温。经过对漂移管温度, 载气压力及喷嘴温度三参数的摸索, 确定蒸发光散射检测器(ELSD)在检测样品中番茄红素含量时的条件为: 漂移管温度: 45℃; 载气压力: 30psi; 喷嘴温度: 40℃; 增益: 1。以番茄红素纯品为参比样品, 比较了 ELSD 与经常使用的紫外—可见光(UV-VIS)检测器在对番茄红素定量检测时的结果。结果表明: 虽然 ELSD 的灵敏度不及 UV-VIS 检测器, 但完全可以在番茄红素的定量检测中应用。同时, ELSD 可以检测出样品中不带发光基团的其它组分, 从而在检测萃取物及产品纯度时有良好的应用前景。

**关键词:** 蒸发光散射检测器; 高效液相色谱; 番茄红素

## Application of Evaporative Light Scattering Detector (ELSD) in Lycopene Quantification by RP-HPLC

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**Abstract:** In this study, lycopene from tomato fruits was separated on HPLC by reversed phase C<sub>18</sub> column under the conditions as: C<sub>18</sub> stationary phase-DIAMONSIL™ column mobile phase-A ratio of acetonitrile to water (9:1) and mobile phase-B ethyl acetate; linear binary elution gradient: mobile phase B increased from 0 to 100% in the first 15 minutes and later kept 100% consequently; flow rate-1.0 ml/min; UV-VI detection wavelength 260~600nm; monitoring wavelength-475nm; column temperature-ambient temperature. The detection conditions applied for this sample of evaporative light scattering detector (ELSD) were: drift temperature-45℃; carrier gas pressure-30psi; sprayer temperature-40℃; and gain-1. After variation in drift temperature, carrier gas pressure and sprayer temperature, the comparison of quantitative results from ELSD and UV-VIS detector was made with the reference of pure lycopene. Data from the comparison of results suggested that ELSD can be applied in lycopene

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