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Short communication

Determination of clopidol residues in chicken tissues by high-performance liquid chromatography–mass spectrometry

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Abstract

A high-performance liquid chromatographic–mass spectrometric (HPLC–MS) method has been developed for determination of clopidol residues in chicken tissues. Samples are extracted with acetonitrile. The extracts are cleaned up on an alumina column followed by an anion-exchange column. The clopidol is separated on a column (150 cm×4.6 mm) of Intertsil by using acetonitrile–water (20:80) as mobile phase. The clopidol was qualitatively identified by molecule mass and determined quantitatively by selected ion monitoring mode at 190 *m*/*z*. The recoveries with RSDs ranged from 91.6±10.1 to 97.3±5.7 at 0.010 to 10.0 mg/kg by spiking three matrices (chicken muscle, liver, and kidney). The limit of detection was 0.005 mg/kg, and the limit of quantification was 0.010 mg/kg.

Keywords: Meat; Food analysis; Clopidol

1. Introduction

Clopidol (3,5-dichloro-2,6-dimethyl-4-pyridinol) is a feed additive used to control coccidiosis in chickens, which is widely used all over the world. Every country has strict regulations on maximum residue limit (MRL) for the clopidol in products of animal origin [1]. The analytical methods of clopidol residue in chicken tissues consist of diazomethane derivatization followed by gas chromatography (GC) [2,3], propionic anhydride derivatization followed by GC [4], acetic anhydride derivatization followed by GC [5] and high-performance liquid chromatography (HPLC) [6,7]. In China, large quantities of chicken are exported each year to Japan and Africa, and the control of clopidol residues in chicken is rather stringent. In our laboratory, clopidol residues are determined by HPLC on a lot-by-lot basis prior to butchering in order to make sure that the export chicken will conform to the MRL stipulations for clopidol of the importing country. If it is detected, it must be confirmed by GC–MS so as to determine if the results are truly positive or falsely positive. To simplify the operation procedures, we report a rapid identification and quantification of clopidol residues in the chicken tissues by HPLC–MS. This method has improved working efficiency and saved the analytical time in comparison with GC methods.

2. Experimental

2.1. Chemicals and solvents

Alumina (neutral, 70–230 mesh) was purchased from Shanghai Five-Four Chemical Reagent Factory (Shanghai, China). Anion-exchange resin (Dowex 1-X8, 100–200 mesh, chloride form) was purchased

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from Crescent Chemical Co., Inc. (NY, USA). It was converted to the acetate form by the procedure of the Analytical Methods Committee [8]. Clopidol (purity 99%) was purchased from Guangzhou Nanfang Scientific Instrument Co. (Guangzhou, China). Acetonitrile and methanol (LC grade) were purchased from Beijing Chemical Factory (Beijing, China).

2.2. Equipment

Waters model 616 HPLC system was coupled to a Finnigan MAT TSQ-7000 quadrupole mass spectrometer. Liquid chromatographic columns: (1) alumina column: a small plug of glass wool was placed at the bottom of the glass column (400×20 mm I.D.) with PTFE stop cock. The glass column was filled to about 1/3 full with methanol, and 15 g alumina was poured in, the solvent was drained to the top of the column. (2) Anion-exchange column: a small plug of glass wool was placed at the bottom of the glass column (200×12 mm I.D.) with PTFE stop cock. The methanol slurry of Dowex exchange resin in acetate form was poured into the glass column to a bed height of 15 mm after settling, and the solvent was drained to the top of the column.

2.3. Sample preparation

All chicken tissue samples were obtained from Qinhuangdao Chia Tai Co. Ltd. Meat Processing Factory (Qinhuangdao, China) and stocked at −18°C. A 10.0 g amount of minced chicken tissue was accurately weighed into a centrifuge tube filled with 20 g anhydrous sodium sulfate. A 50 ml volume of acetonitrile was added and the mixture was homogenized for 2 min at 10 000 rpm, and then centrifuged for 5 min at 3000 rpm. The transparent extracts in the centrifuge tube were poured onto the alumina column, which was placed just above the anion-exchange column. The sediments were re-homogenized in the centrifuge tube with 50 ml acetonitrile and then re-centrifuged. The re-extracts were combined onto the alumina column, the extracts were let go through both clean-up columns at 3 ml/min, and then the centrifuge tube was rinsed with 20 ml methanol and the washes were used to rinse both clean-up columns successively, the eluate was discarded. The alumina column was removed, clopidol was eluted with 20 ml 0.5% acetic acid–methanol from the anion-exchange column into a 100 ml evaporating flask. This was evaporated to dryness on a rotary evaporator at 60°C, and the residues were re-dissolved in 1.0 ml methanol for HPLC–MS analysis.

3. Results and discussion

3.1. Identification and quantification of clopidol

HPLC–MS acquisition parameters of the proposed method are shown in Table 1.

The full scan negative ionization mass spectra of

Table 1
HPLC–MS analytical conditions

Description	Parameter
Reversed-phase column	Intertsil ODS (150×4.6 mm)[a]
Mobile phase	Acetonitrile–water (20:80)
Flow rate	0.8 ml/min
Interface	Atmospheric pressure chemical ionization
Scan range	180–200 aum
Main molecule ions	m/z 190, m/z 192, m/z 194
Target ion	m/z 190
Corona voltage	1.8 kV
Corona current	5.0 μA
Capillary temperature	220°C
Electron multiplier	1400 V
Vaporizer temperature	400°C

[a] Supplied by GL Sciences Tokyo, Japan.

clopidol are shown in Fig. 1A. The three molecule ion peaks ([M-H]$^-$) of clopidol are at m/z 190, 192 and 194, respectively. There are no fragment ion peaks as APCI is a soft ionization mode. The clopidol can be identified qualitatively by molecular mass, and determined quantitatively by selected ion monitoring mode at m/z 190 in order to raise sensitivity and selectivity in this experiment.

3.2. Method's limit of detection (LOD) and limit of quantification (LOQ)

The linear range of the five-point working calibration curve is from 1.0 to 20.0 ng. The regression coefficient (r) is ≥0.994. The LOD of this method was 0.005 mg/kg. The signal-to-noise ratio of clopidol was actually considerably greater than five times the noise. It was conservatively established pending future applications of the method to more highly matrix effected commodities. For 10 blank chicken samples, each one fortified at 0.010 mg/kg clopidol, the average recovery was 80% with an RSD of 12.5%, the LOQ of this method was 0.010 mg/kg for clopidol, which is the lowest MRL specified in the world. Total-ion chromatograms from the blank sample and fortified sample at LOQ

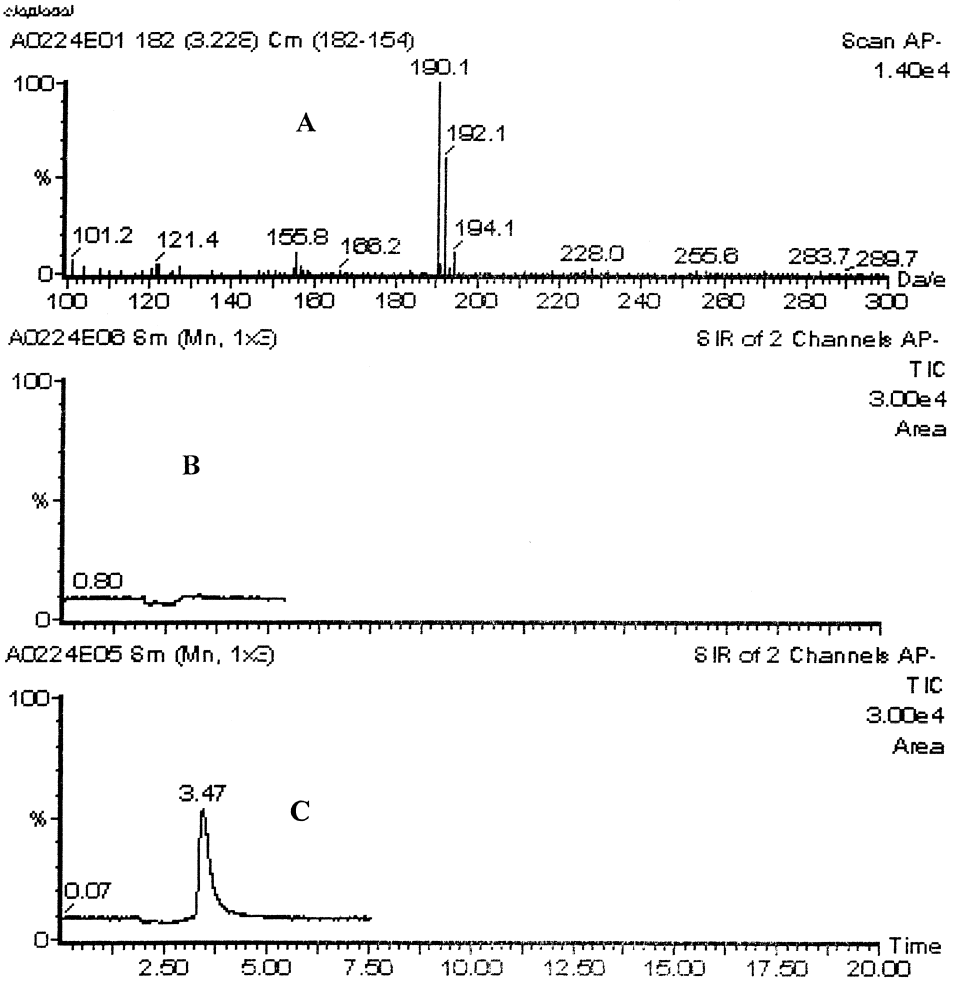

Fig. 1. Mass ion spectra from clopidol standard (A) and total-ion chromatograms from blank sample (B), fortified sample of chicken at LOQ (C).

Table 2
Analytical results of recoveries and reproducibilities (n=5)

Chicken			Liver			Kidney		
Added (mg/kg)	Mean recovery (%) ±RSD (%)		Added (mg/kg)	Mean recovery (%) ±RSD (%)		Added (mg/kg)	Mean recovery (%) ±RSD (%)	
	MS	UV		MS	UV		MS	UV
0.010	93.3±16.4	94.3±12.0	0.30	96.7±6.9	100.0±5.8	0.30	94.5±7.4	88.7±4.0
0.10	97.3±5.7	83.0±2.1	3.0	91.7±2.9	92.6±2.6	3.0	91.6±10.1	91.9±7.8
1.0	92.0±6.0	87.3±3.7	10.0	93.7±3.1	93.3±3.2	10.0	92.1±6.8	92.3±3.8

are shown in Fig. 1B and C. These experiments demonstrated that no interfering peaks were observed on chromatograms of chicken samples, the efficiencies of the extraction and clean-up of the proposed method were acceptable, and the analytical results were reliable.

3.3. Comparison of method efficiency between HPLC–MS and HPLC–UV

Recovery studies were carried out with three different kinds of samples at three different fortified levels from 0.010 to 10.0 mg/kg. The samples were analyzed by the method described above and our previously developed HPLC–UV method [6]. The analytical results from fortified muscle, liver, and kidney samples are given in Table 2.

These recoveries and reproducibilities determined by methods are satisfactory and suitable for residue analysis. The reproducibility of the HPLC–UV method is better than that of HPLC–MS, HPLC–MS is more reliable more than HPLC–UV, the two methods have been applied for routine analysis of clopidol residue in chicken tissues.

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