ELSEVIER

Journal of Chromatography B, 728 (1999) 273–277

JOURNAL OF CHROMATOGRAPHY B

Short communication

High-performance liquid chromatography determination of residue levels on chicken carcasses treated with cetylpyridinium chloride

Xiang Zhou, Andronica Handie, Hamid Salari, E. Kim Fifer, Philip J. Breen, Cesar M. Compadre*

Food Safety Consortium Group and Department of Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Slot 522, 4301 W. Markham, Little Rock, AR 72205, USA

Received 6 August 1998; received in revised form 25 February 1999; accepted 26 February 1999

Abstract

Cetylpyridinium chloride (CPC) has been found to be effective in reducing contamination of chicken carcasses from a variety of microorganisms, including *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Listeria monocytogenes*, and *Staphylococcus aureus*. A procedure has been developed to determine residue levels on chicken carcasses after CPC treatment. For the analysis, chicken carcasses were extracted with 95% ethanol. The CPC concentration in the extract was measured by high-performance liquid chromatography (HPLC) with ultraviolet detection using dodecylpyridinium chloride (DPC) as an internal standard. The method was validated in the concentration range of 3–200 μg/ml CPC in ethanolic extract. This assay is rapid, precise, and accurate. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Cetylpyridinium chloride

1. Introduction

Food-borne illness resulting from microbial contamination is a major concern in the United States and the world. There is great interest in developing effective methods for microbial decontamination in the poultry and meat processing industry and in regulatory agencies. A variety of chemical and physical approaches have been studied to reduce and eliminate microorganisms from food products [1–5]. However, the existing techniques are not completely effective in removing microorganisms from poultry and meat tissues and some of them may adversely affect flavor, color, and/or texture of the products.

It has been found that CPC (Fig. 1a), which has been safely used in oral hygiene products for decades, is very efficient in reducing microbial contamination in poultry tissues [6–8]. This compound is more effective in removal of various microorganisms from chicken carcasses than chlorine, which is currently used in the poultry industry.

In order to develop a practical method for the microbial decontamination of chicken carcasses using CPC treatment, a method for measurement of residue levels on treated carcasses is needed. Methods for the determination of CPC in mouthwash [9], saliva [10], cosmetics [11], antiseptics [12], pharmaceuticals [13–15], and some other matrices [16–19]

*Corresponding author. Tel.: +1-501-686-6493; fax: +1-501-686-6057.

0378-4347/99/$ – see front matter
PII: S0378-4347(99)00100-0

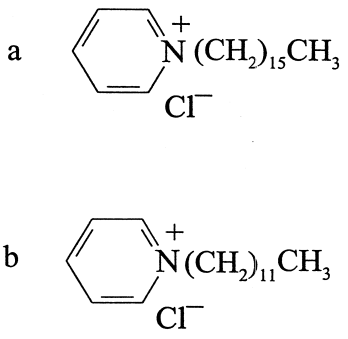

Fig. 1. Chemical structures of (a) cetylpyridinium chloride and (b) dodecylpyridinium chloride.

have been reported. The techniques used include HPLC [10,11,14,16,17], thin-layer chromatography [12], pyrolysis gas chromatography [18], colorimetry [13], spectrophotometry [15], and adsorptive voltammetry [19]. Currently, there is no fully validated assay available for determining CPC levels in biological tissues. In the present work, a method to measure CPC residue on chicken carcasses is reported.

2. Experimental

2.1. Chemicals and materials

CPC was purchased from Zeeland Chemicals, (Zeeland, MI, USA). DPC (Fig. 1b) and tetramethylammonium hydroxide pentahydrate (TMAHP) were obtained from Aldrich (Milwaukee, WI, USA). HPLC-grade methanol and water were purchased from Fisher Scientific (Pittsburgh, PA, USA). Alcohol USP (95% ethanol and 5% water) was obtained from Aaper Alcohol (Shelbyville, KY, USA).

2.2. HPLC conditions

HPLC analyses were performed using an Waters Corp. (Milford, MA, USA) system consisting of Waters 600E Multisolvent Delivery System, Waters 490E Programmable Multiwavelength Detector, Waters 746 Data Module, and Waters 600E System Controller. The column (Alltima cyano, 250×4.6 mm, 5 μm) and the guard cartridge (Alltima cyano, 7.5×4.6, 5 μm) were obtained from Alltech Associates (Deerfield, IL, USA). Mobile phase was methanol: 0.008 *M* TMAHP–acetic acid buffer, pH 3.6 (37:63), at a flow rate of 2 ml/min. The molarity of acetic acid in the buffer was 0.14 *M*. Ultraviolet absorbance detection was at 260 nm. Each run was completed in 9 min.

2.3. Assay

Frozen chicken carcasses (−70°C) were weighed and then thawed to room temperature. Each carcass was agitated with 900 ml of 95% ethanol in a closed plastic bag at 60°C for 1 h. After cooling to room temperature, the extract was transferred to a 1-l volumetric flask, and diluted with 95% ethanol to 1 l. Five-ml aliquots of the extract were spiked with 135 μg (56 μl) of ethanolic DPC as internal standard and centrifuged at 15 000 *g* for 8 min. The supernatants (20 μl) were injected into HPLC for analysis.

2.4. Calibration

Five-ml aliquots of the blank extract (ethanolic extract of CPC-free chicken carcasses) were spiked with 66 μl of ethanolic CPC standards to give concentrations of 3.13, 6.25, 12.5, 25, 50, 100 and 200 μg/ml. Ethanolic DPC (56 μl, 135 μg) was added to each sample. The samples were analyzed as described above (Section 2.3). Linear regression was performed between the peak–area ratio of CPC to DPC versus CPC concentration.

2.5. Extraction recovery

Ten ml of aqueous CPC (6.09 and 30.1 mg) were evenly applied to the surface of CPC-free chicken carcasses using a Pasteur pipette. The carcasses were then stored in a freezer at −70°C until analysis. The carcasses were thawed to room temperature, extracted, and measured for CPC as described above (Section 2.3). The extraction recovery was calculated as the ratio of measured to added CPC.

2.6. HPLC peak purity

To test peak purity, the UV-absorbance ratio between 245 and 260 nm was measured for peaks

from ethanolic CPC standards and chicken extracts. The HPLC conditions were the same as above (Section 2.2) except that the absorbance ratio instead of single wavelength absorption was detected.

2.7. Assay application

Five chicken carcasses weighing 1.49–1.53 kg were immersed in 0.0125% aqueous CPC for 60 s, placed in an ice water bath for 45 min, and then stored under refrigeration (−70°C). The residual CPC levels in the carcasses were analyzed as described above (Section 2.3).

3. Results and discussion

3.1. Optimization of experimental conditions

Internal standard method is employed in this assay for reduction of possible analytical errors resulting from change in the sensitivity of HPLC detector, inaccurate injection volume, and other experimental variations. Cyano stationary phase has been used for HPLC determination of CPC as a trace contaminant in the polysaccharide, chondroitin sulfate previously [16]. In the present case, the cyano stationary phase provides better separation for the analysis than C_{18} phase, which retains CPC very strongly. The utilization of the buffer for the mobile phase is important since CPC peak exhibits considerable tailing when the buffer is not used. CPC's strongest UV absorption above the buffer cut-off is at 260 nm, which has been monitored in HPLC analysis to give the highest sensitivity.

3.2. Specificity

Representative chromatograms for a blank extract, blank extracts spiked with CPC and/or DPC, and an extract of a CPC-treated chicken carcass spiked with DPC are shown in Fig. 2. The retention times for DPC and CPC are about 3.8 and 6.5 min, respectively. In these regions, there is no significant interference from chicken extractives. The specificity was also confirmed by measurement of UV-absorbance ratio: the absorbance ratio between 245 and 260 nm for CPC peak from ethanolic CPC standards is 0.44, which is the same as the absorbance ratio between

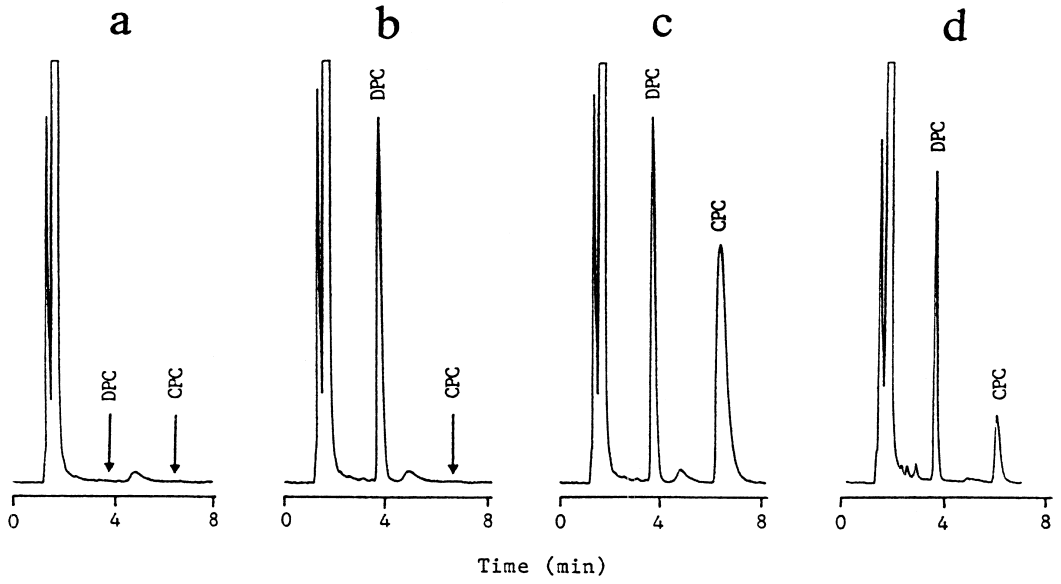

Fig. 2. High-performance liquid chromatograms of: (a) blank extract; (b) blank extract spiked with DPC at 27 μg/ml; (c) blank extract spiked with DPC and CPC at 27 and 50 μg/ml, respectively; and (d) extract of a CPC-treated chicken carcass spiked with DPC at 27 μg/ml (measured CPC concentration: 13.6 μg/ml in the extract or 9.06 mg/kg in the carcass).

Table 1
Inter-assay precision and accuracy for the determination of CPC in spiked chicken carcass extract ($n=5$)

Spiked conc. (μg/ml)	Measured conc. (μg/ml) (mean±S.D.)	C.V. (%)	Relative error (%)
3.57	4.19±0.314	7.5	17.4
91.3	91.2±1.17	1.3	−0.1
183	184±1.95	1.1	0.5

these two wavelengths for the corresponding peak from chicken extracts.

3.3. Calibration and linearity

A seven-point calibration graph was obtained by plotting the peak–area ratio for CPC to DPC versus CPC concentration. Over the concentration range of 3–200 μg/ml, the linearity is satisfactory as shown by the equation: $y=0.0311x-0.0348$, where x is the concentration and y is the peak–area ratio. The standard deviations for the slope and the intercept are 0.00035 and 0.00662, respectively. The correlation coefficient (r^2) is 0.9997±0.00029 (quintuplicate, $n=5$).

3.4. Precision and accuracy

The inter- and intra-assay precision and accuracy were determined by analyzing replicate ($n=5$) blank extracts spiked with CPC at 3.57, 91.3 and 183 μg/ml and with the internal standard. As shown in Tables 1 and 2, the error was less than 3% for the medium and higher concentrations. The inter-assay error for the lower concentration, 3.57 μg/ml, was 17.4%, which was less than the maximum acceptable error, 20%. The coefficient of variation (C.V.) was less that 9% for the concentrations tested.

Table 2
Intra-assay precision and accuracy for the determination of CPC in spiked chicken carcass extract ($n=5$)

Spiked conc. (μg/ml)	Measured conc. (μg/ml) (mean±S.D.)	C.V. (%)	Relative error (%)
3.57	3.78±0.297	8.1	5.9
91.3	93.7±0.859	0.9	2.6
183	188±3.42	1.8	2.7

3.5. Limit of quantitation

The limit of quantitation in ethanolic extracts, the lowest concentration that can be determined with acceptable precision (C.V.<20%) and accuracy (error<20%), was 3.13 μg/ml [20,21]. Under the conditions used the limit of quantitation in chicken carcasses was about 3 mg/kg. It is anticipated that the CPC residual in treated chicken carcasses will be in the level of 5–20 mg/kg. Therefore this assay is adequate for the analysis.

3.6. Extraction recovery

The extraction recovery were 95.3±6.5 and 84.1±4.7% for the chicken carcasses applied 6 and 30 mg of CPC, respectively ($n=5$).

3.7. Assay application

The residual CPC levels in five chicken carcasses treated by immersion in 0.0125% CPC solution for 1 min were in the range of 8–15.7 mg/kg chicken as determined by the HPLC assay. The average residue was 11.7±3.05 mg/kg.

4. Conclusions

An HPLC assay for determination of CPC residual levels on chicken carcasses has been developed. The assay employs 95% ethanol to efficiently extract CPC from chicken carcasses. The interferences from chicken extractives are well separated from CPC and the internal standard DPC by HPLC. The method is rapid, reproducible, and accurate.

Acknowledgements

This research was supported by grants from the Arkansas Science and Technology Authority, USDA/CSREES through the Food Safety Consortium, and USDA/NRICGP (grant number: 9802739).

References

[1] J. Giese, Food Technol. 47 (1993) 110.
[2] H.S. Lillard, Poultry Sci. 59 (1980) 1761.
[3] H.S. Lillard, J.E. Thomson, J. Food Sci. 48 (1983) 125.
[4] Y. Li, J.W. Kim, M.F. Slavik, C.L. Griffis, J.T. Walker, H. Wang, J. Food Sci. 59 (1994) 23.
[5] A.L. Izat, M. Colberg, R.A. Thomas, M.H. Adams, C.D. Driggers, J. Food Qual. 13 (1990) 295.
[6] P.J. Breen, C.M. Compadre, E.K. Fifer, H. Salari, D.C. Serbus, D.L. Lattin, J. Food Sci. 60 (1995) 1191.
[7] P.J. Breen, H. Salari, C.M. Compadre, J. Food Protection 60 (1997) 1019.
[8] D. Lattin, P. Breen, C.M. Compadre, K. Fifer, H. Salari, M. Slavik, US patent 5,366,983 (1994).
[9] S. Pinzauti, V.D. Piaz, E.L. Porta, G. Pisaturo, J. Pharm. Belg. 31 (1976) 485.
[10] A.E. Collins, P.B. Deasy, J. Pharm. Sci. 79 (1990) 116.
[11] B. Wyhowski de Bukanski, Int. J. Cosmet. Sci. 9 (4) (1987) 193.
[12] J. Paesen, I. Quintens, G. Thoithi, E. Roets, G. Reybrouck, J. Hoogmartens, J. Chromatogr. A 677 (1994) 377.
[13] S.S. Zarapkar, R.V. Rele, V.M. Shah, Indian Drugs 24 (1987) 481.
[14] P. Linares, M.C. Gutierrez, F. Lazaro, M.D. Luque de Castro, M. Valcarcel, J. Chromatogr. 558 (1991) 147.
[15] T. Sakai, N. Ohno, Anal. Sci. 7 (1991) 297.
[16] R.C. Meyer, L.T. Takahashi, J. Chromatogr. 280 (1983) 159.
[17] R.B. Taylor, S. Toasaksiri, R.G. Reid, D. Wood, Analyst 122 (1997) 973.
[18] H. Binder, W. Krainer, W. Lindner, Arch. Pharm. 319 (1986) 642.
[19] S.B. Adeloju, S.J. Shaw, Electroanalysis 6 (1994) 645.
[20] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309.
[21] G. Flesch, Ch. Mann, P.H. Degen, J. Chromatogr. B 696 (1997) 123.