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High-Performance Liquid Chromatographic Determination of Oxytetracycline Residue in Cured Meat Products

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The development of a sensitive automated method for residue control of oxytetracycline (OTC) in cured meat (soudjouk) is described. The principle steps involve the extraction of OTC from cured meat in the presence of citric acid with nitric acid, methanol and de-ionized water, and then the determination by HPLC. In HPLC, Hypersil BDC C18 column was used and OTC was separated at 24°C using a mobile phase of H_2O (pH=2.10 with H_2SO_4): ACN (85:15, v/v) at a flow rate of 1.5 ml/min. A variable wavelength detector was set at 360 nm. The detection limit of the method was calculated at 8 ng/g and the minimum detectable quantity was found as to be 40 mg/ml. The statistical evaluation demonstrated high absolute recoveries of from 78 to 100% for OTC. The method was also used with 10 different cured meat samples marketed in Turkey, and in 7 of them the OTC residue was found to be higher than the tolerance levels accepted by the EU, FDA and Turkish Ministry of Agriculture.

Key Words: Oxytetracycline, HPLC, soudjouk, cured meat

Introduction

Antibiotics are widely used in food-producing animals for the treatment of disease and as dietary supplements. They may be administered orally as food additives or directly by injection. The use of antibiotics may result in drug residues in the meat, especially if they are not used according to label directions. The presence of antibiotic residues in meat, milk, etc. may cause allergic reactions in sensitive individuals[1].

The tetracyclines, among the first of the antibiotics to become available 50 years ago, are still widely used. Tetracyclines have bacteriostatic activity against a wide variety of pathogens that are responsible for many common and some exotic infections. These antibiotics are widely used for the treatment of bovine mastitis and are added at subtherapeutic levels to cattle feeds for prophylaxis[2].

Oxytetracycline (OTC), *[4S-4a,4aα,5a,5aα,6b,12aα)]-4-(dimethylamino)-,4,4a,5,5a,6,11, 12a-octahydro-3,5,6,10,12,12a-hexahydoxy-6-methyl-1,11-dioxo-2-naphtacenecarboxamide* (Fig.1), is commonly used for the

prevention and/or treatment of diseases in livestock production. As a feed additive in subtherapeutic doses, it contributes to the maintenance of optimal health and thus promotes growth in food-producing animals[3].

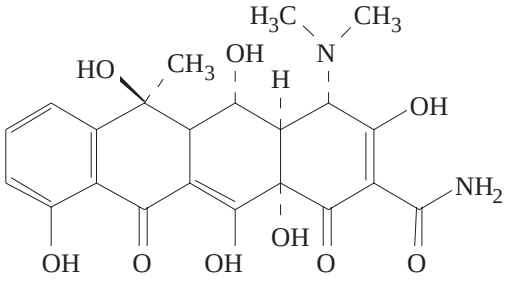

Figure 1. Structural formula of oxytetracycline

However, the use of this compound may result in residues in animal derived food products, especially if proper withdrawal times for treated animals have not been used[3].

These residues may pose a health threat to consumers, depending on the type of food and the amount of residue present. For this reason, regulatory agencies have established maximum legal tolerance levels for this drug in animal derived food products[4].

OTC residues that exceed the tolerance level may be of toxicological concern. Some individuals may have an allergic reaction to these compounds, and/or resistance by some bacteria may be induced. Treatment with OTC during the second month of pregnancy presents a teratogenic risk to the fetus[5,6]. As undesirable side-effects, OTC not only discolors the primary and permanent teeth but also causes hypoplasia in developing teeth when administered to infants, mothers during the last two trimesters of pregnancy and children under 12 years of age[7,8,9]. It has also been suggested that discoloration caused by tetracyclines occurs in adult dentition[10].

Children receiving OTC may develop brown discoloration of the teeth. The risk of this untoward effect is highest when OTC is given to neonates and babies prior to the first dentition. However, pigmentation of the permanent dentition may develop if the drug is given between the ages of 2 months and 12 years, when these teeth are being calcified. An early characteristic of this defect is a yellow fluorescence of the dental pigment, which has an ultraviolet spectrum with an absorption peak at 270 nm. The deposition of the drug in the teeth and bones is probably due to its chelating property and the formation of a tetracycline-calcium orthophosphate complex. As time progresses, the yellow fluorescence is replaced by a nonfluorescent brown color that may represent an oxidation product of the antibiotic, the formation of which is hastened by light. Treatment of pregnant women with OTC may also produce discoloration of the teeth in their children[11].

The Turkish Ministry of Agriculture, the EU and the FDA have set levels of concern for residues of OTC in meat/flesh at 0.1 mg/Kg [12,13].

Methods used to determine OTC residue levels include microbiological[14], mass spectrometric[15], and HPLC [16−20].

Recently, for OTC in cured meat (soudjouk) products marketed and consumed in Turkey, we have developed an extraction and determination technique with high recoveries and minimal background interferences.

Experimental

Reagents

Standard Merck Oxytetracycline HCl was obtained from Pfizer Inc./Turkey. Certified grade citric acid was obtained from Merck Inc. Methanol, acetonitrile and nitric acid were obtained from Carlo Erba and 0.45 μm Nylon filters were obtained from Waters Assoc. Other chemicals were of analytical or of HPLC grade.

HPLC

The system consisted of the following components: A Hewlet Packard Series 1100 Liquid Chromatograph including a 7725 rheodyne injector (20 μl loop), HP UV-Vis detector, vacuum degasser, gradient pump module and column compartment oven.

Chromatographic Conditions

Isocratic separation was achieved using a Hypersil BDS C_{18} (5 mm, 250×4 mm) column supplied by HP (Germany). The mobile phase consisting of distilled water (pH = 2.1 with H_2SO_4): Acetonitrile, 85:15 (v/v), was pumped at a flow rate of 1.5 ml/min. The analytes were detected at 360 nm using a setting of 0.01 A.U.F.S. The injected volume was 20 μl and chromatography was performed at 24°C.

Extraction

2 g cured meat product (soudjouk) was homogenized in a blender for 2 min and then 0.1 g citric acid was added. To this mixture, 1 ml nitric acid (30%), 4 ml methanol and 1 ml deionized water were added, respectively. The suspension with solid particles was put in a vortex for good mixing, kept in an ultrasonic bath for 15 min and then centrifuged for 10 min at 5300 rpm. After filtering through a 0.45 μm nylon filter, 20 μl of solution was injected into HPLC for analysis.

Standard Curve

Calibration standards were prepared using concentrations of 0.25, 0.5, 1.5, 2.25, 3.0 and 6.0 mg/L of OTC in eluent. These standards were prepared from the daily prepared stock solution and treated as indicated above.

Detection Limit (DL)

Detection Limit (DL) is defined as the concentration of OTC that produces an analytical signal equal to thrice the standard deviation of the background signal and calculated as 8 ng/g. By definition the relative standard deviation at the DL is 50%. Thus measurements are normally made at concentrations at least a factor of ten above the DL[21] and found as 40 mg/ml.

Results and Discussion

A rapid, simple and specific method for the determination of OTC in cured meat products has been developed, and used with products marketed in Turkey. The method involves the extraction of OTC from cured meat products and the determination by HPLC. The method was used with ten different cured meat

products marketed in Turkey and the tabulated results of the OTC determination by HPLC following the extraction step are given in Table 1.

Table 1. Results of OTC in soudjouk at a dose of mg /Kg

Product Code	OTC Concentration (mg/Kg) (n=5)
A	3.6
B	Below MDQ
C	0.48
D	1.92
E	5.34
F	0.42
H	Below MDQ
I	Below MDQ
K	1.26
L	0.78

The results indicate that seven out of ten cured meat products contain OTC higher than the tolerance level announced by the EU, FDA and Turkish Ministry of Agriculture. The mean recovery of the method was found to be 78-100%, by adding known amounts of OTC (Table 2).

Table 2. Recovery results obtained in cured meat (soudjouk) products sold in Turkey.

Product code (mg/L)	OXYTETRACYCLINE ADDED (mg/L)	FOUND % (n=5)	RECOVERY
A	0.50	0.50	100
B	0.50	0.49	98
C	0.50	0.39	78
D	0.50	0.40	80
E	0.50	0.48	96
F	0.50	0.44	88
H	0.50	0.48	96
I	0.50	0.46	92
K	0.50	0.40	80
L	0.50	0.47	94

As can be seen from the accompanying data, excellent linearity and reproducibility were obtained for the detection of a number of spiked OTC samples in extracted cured meat. A sample chromatogram output for a spiked sample is shown in Figure 2 . At the lowest concentration used, 0.01 mg/μl, it was observed that the signal to noise (peak to peak amplitude noise) ratio was 70:1 for OTC.

It is therefore demonstrated that the above method is a highly sensitive, selective technique for OTC analysis, and eight of the cured meat product samples exceed current legislation for the tolerance level in meat.

The proposed method is reproducible, rapid and simple to use in assaying soudjouk and also can serve as an identification and stability indicating assay for OTC.

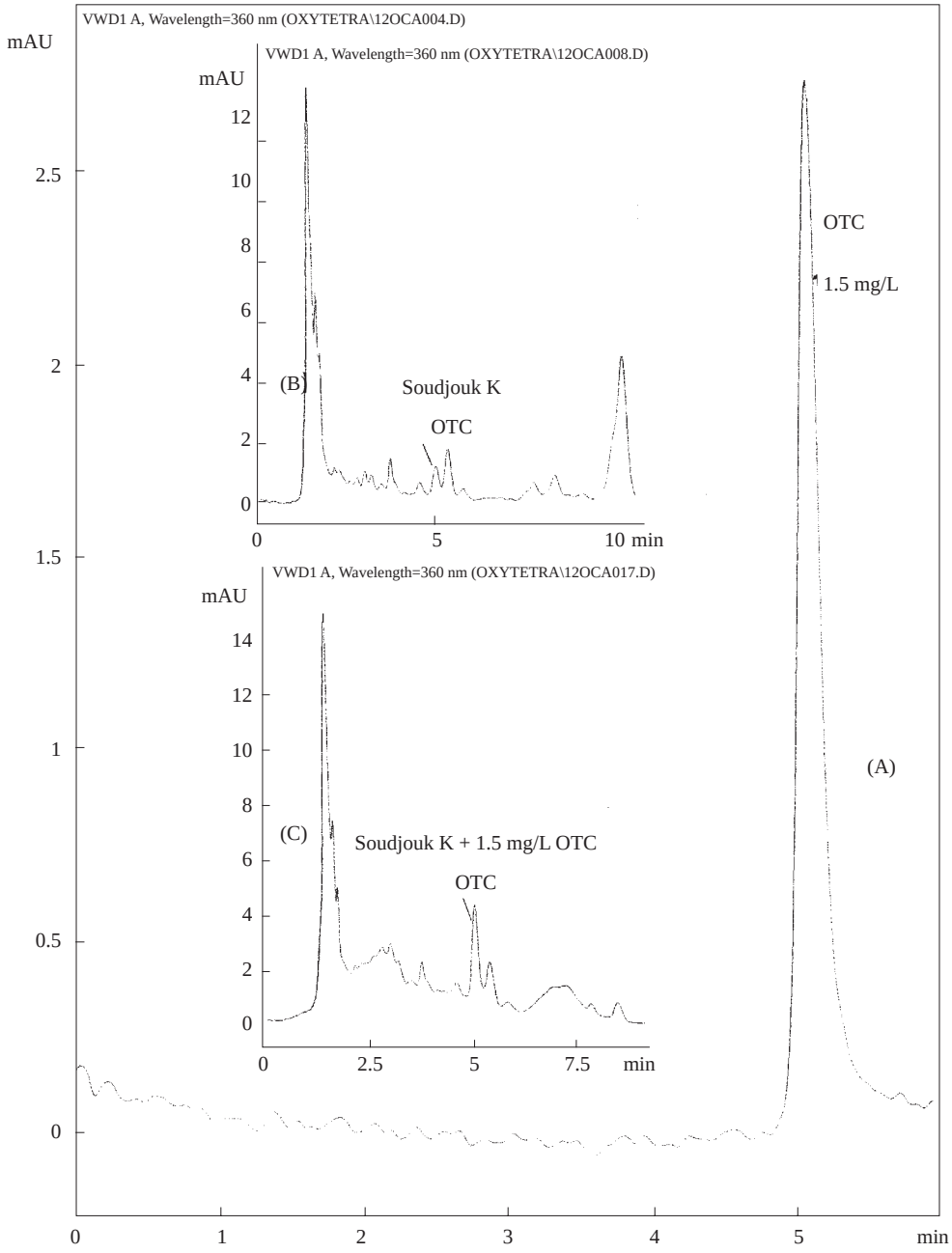

Figure 1. Chromatogram obtained from standard and spiked soudjouk sample. (A) Standard OTC (1.5 mg/L), (B) extracted soudjouk sample and (C) spiked (1.5 mg/L OTC) soudjouk sample.

References

1. F.J. Schenck and P.S. Callery, **J. Chromatog. A 812,** 99-109 (1998) "Chromatographic methods of analysis of antibiotics in milk"
2. J.D. Smilack, **Mayo Clinic Proceedings 74(7)** 727-9 (1999) "The Tetracyclines"

3. A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short, S.A. Barker, **J. Assoc Anal. Chem. 73(3),** pp.379-84, 1990 "Matrix Solid-Phase Dispersion (MSPD) Isolation and Liquid Chromatographic Determination of Oxytetracycline and Tetracycline and Chlorotetracycline in Milk."
4. U.S. Department of Agriculture, Food Safety and Inspection Service. Compound Evaluation and Analytical Capability. National Residue Program, P.J. Brown (Ed). USDA, Washington DC, 1988.
5. A.E. Czeizel, M. Rockenbauer and J. Olsen, **European J. of Obstetrics and Gynecology and Reproductive Biology, 81** 1-8 (1998). "Use of antibiotics during pregnancy".
6. A.E. Czeizel and M. Rockenbauer, **European J. of Obstetrics and Gynecology and Reproductive Biology, 88,** 27-33 (2000). "A population-based case-control teratologic study of oral OTC treatment during pregnancy".
7. W. Ainley and J.E.F. Reynolds, **Martindale: The Extra Pharmacopoeia,** 27^{th} Edition, pp. 279, The Pharmaceutical Press, London, 1977.
8. J.G. Walton, J.W. Thompson, R.A. Seymour, Textbook of Dental Pharmacology and Therapeutics, pp-123-124, Oxford University Press, Oxford, 1994,
9. B.S. Requa-clark, S.V. Holroyd, **Applied Pharmacology for the Dental Hygienist, Mosby, St. Louis,** pp-115-118, 1995.
10. S. Tanase, H. Tsuchiya, J. Yao, S. Ohmoto, N. Takagi, S. Yoshida, **J. Chomatogr. B. 706,** 279-285, (1998) "Reversed-Phase ion-pair chromatographic analysis of tetracycline antibiotics: Application to discolored teeth"
11. W. R. Theodore, S.N. Alan, T. Palmer, **Goodman and Gilman's The Pharmacological Basis of Therapeutics,** 8^{th} Edition, Chapter 48, "Antimicrobial Agents", pp1122, Pergamon Press, USA, 1990.
12. FDA, Agriculture Drugs, Hazards and Control Guide, 1st ed. 103-116. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food safety and Applied Nutrition Office, Washington, DC. 1996.
13. S. Kaya, "Türkiye'de Veteriner ilaçları üretimi, pazarlanması, güvenli kullanımı ve kalıntı sempozyumu" **Türk Toksikoloji Derneği ve VİSAD,** pp.152-174, 1994.
14. J.L. Grondel, J.F.M. Nouws, M. DeJong, A.R. Schutte, F. Driessens. **J. of Fish Diseases, 10,** 153-163, (1987). "Pharmacokinetics and tissue distribution of oxytetracycline in carp, Cyprinus carpio L., following different routes of administration".
15. A. M. Kamel, P. R. Brown, B. Muson., **Anal. Chem, 71(5),** 968-977, (1999). "Electrospray ionization of Tetracycline, Oxytetracycline, Chlorotetracycline, Minocycline and Methacycline".
16. M.A. Radwan, I.Y. Zaghloul, A.I. Radwan, Z.H. Aly, **Int. J. Pharm. 160(1),** 91-97, (1998). "Multiple-dose pharmokinetics of long-acting OTC in Brucella melitensis-infected sheep".
17. P.J. Kijak, C.V. Cope, W.M. Pedersoli., **J. Assoc. Anal. Chem. 82(6)** 1329-33, (1999). "Determination of OTC in bovine kidney and medicated milk replacer by liquid chromatography".
18. K.L. Anderson, W.A. Moats, J.E. Rushing, D.P. Wesen, M.G. Papich, **American J. of Veterinary Research 56(1),** 70-77 (1995). "Potential for oxytetracycline administration by three routes to cause milk residues in lactating cows, as detected by radioimmunoassay and HPLC test methods".
19. G. Boatta, A. Pau, M. Palomba, L. Arenare, R. Cerri, **J. Pharm. Biomed. Anal., 20(1-2),** 321-326, (1999). "Monitoring of oxytetracycline in ovine milk by HPLC".
20. A. Posyniak, J. Zmudzki, S. Semeniuk, J. Niedzielska, R. Ellis. **Biomed. Chromatog., 12(5),** 294-299, (1998). "Determination of tetracycline residues in animal tissues by liquid chromatography".
21. J.D. Ingle Jr and S.R. Crouch, "Spectrochemical Analysis" Prentice-Hall U.K. Ltd., 1988.