

Determination of Oxytetracycline, Penicillin G and Sulphadimidine Residues in Cow Milks in Bursa

H. Hüseyin ORUÇ* Songül SONAL**

Geliş Tarihi: 25.03.2005

Kabul Tarihi: 04.05.2005

Summary: Oxytetracycline, penicillin G (benzylpenicillin) and sulphadimidine levels were analysed by high-performance liquid chromatography (HPLC) methods in 25 raw cow milk samples, which were collected from plain and mountain villages during March-April 2003 in Bursa province. None of the antibacterials were detected in the milk samples suggesting that the milk samples are not a risk for consumer health.

Key Words: Oxytetracycline, penicillin G, sulphadimidine, cow milk.

Bursa'daki İnek Sütlerinde Oksitetrasiklin, Penisillin G ve Sulfadimidin Kalıntılarının Belirlenmesi

Özet: Oksitetrasiklin, penisillin G (benzilpenisillin) ve sulfadimidinin düzeyleri, 25 çiğ inek sütünde, yüksek performanslı likid kromatografi (HPLC) ile ölçüldü. Numuneler, 2003 yılı Mart ve Nisan aylarında Bursa'nın ova ve dağ köylerinden toplandı. Numunelerin hiçbirinde bu antibakteriyeller tespit edilemedi ve bu numunelerin tüketici sağlığı açısından bir risk oluşturmadığı kanısına varıldı.

Anahtar Kelimeler: Oksitetrasiklin, penisillin G ve sulfadimidinin, inek sütü.

Introduction

Antibacterial drugs such as oxytetracycline, penicillin G (benzylpenicillin) and sulphadimidine are routinely used in veterinary medicine for prevention and control of disease in Turkey. Antibacterial drugs use may produce residues in milk and subsequently, the induction of allergic reaction in humans, as well as resistance in pathogen bacteria, which may result in health problems^{9,10}. Oxytetracycline is applied to the prevention or treatment of bronchopneumonia, mastitis and metritis in cows¹³. As a result, there is concern that residues of the compound may be retained in the milk. To prevent any health problems with consumers, World Health Organization

(WHO), US Food and Drug Administration (FDA), European Union (EU) and Turkey (Turk Gıda Kodeksi/Turkish Food Codex) have established its maximum residue limit (MRL) in milk as 0.1 µg/ml^{4,6,16}. The penicillins are widely used to treat or prevent local and systemic infections of farm animals caused by susceptible bacteria in Turkey. The use of penicillins as intramammary administration to treat or prevent bovine mastitis is widespread¹³. MRL was set at 4µg/ml for penicillin G in milk in EU and Turkey^{3,16}. The sulfonamides are commonly used to treat or prevent acute systemic or local infections in animals, sulphadimidine is also used in farm animals in Turkey. Mastitis, metritis, actinobacillosis, respiratory infections and coccidiosis in animals are

* Assoc. Prof. U.U. Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Bursa, Turkey.

** Prof. Dr. U.U. Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Bursa, Turkey.

treated with sulfonamides¹³. MRL were set at 100 µg/l for sulphonamides in milk in EU and Turkey^{12,16}.

There are some studies about tetracyclines, penicillins and sulphonamides levels in the milk in Turkey^{5,11,15} and other countries^{1,7,8,14,17}, and sometimes these antibacterial agents can be found in the milk samples^{5,17}. In Turkey, there are not enough data on the factual contamination of these agents in the milk. For this reason, the aim of the study was to data on the presence of oxytetracycline, penicillin G and sulphadimidine in milk consumed in Bursa and to discuss the probable risks of these agents residues.

Material and Method

The research materials consisted of 25 raw milk samples that were collected from plain and mountain villages during March–April 2003 in Bursa province. All samples were transported under chilled conditions to the laboratory, and milk samples were stored at -40 °C. The samples were analyzed during March-May 2004 for oxytetracycline HCl, penicillin G and sulphadimidine sodium by using high-performance liquid chromatography (HPLC) (Shimadzu, LC-10AT) with a photo-diode array (Shimadzu, SPD-M10A) detector. Penicillin G standard (procaine salt) was obtained from Sigma (St. Louis, MO, USA), oxytetracycline HCl and sulphadimidine sodium standards were obtained from Sanofi-DIF (Istanbul, Turkey). A simple method for determination of residual oxytetracycline in milk by HPLC was developed, according to the procedure described by Furusawa⁷. Extraction was made with 20% (v/v) trichloroacetic acid (TCA) from milk and filtered through a 0.45-µm disposable syringe filter unit. A C-8 column (Biochemmock, 7µm, 250x 4 mm I.D.) and a mobile phase of acetonitrile-acetic acid-water (28:4:68, v/v/v) with a photo-diode array (UV detection at 354 nm) detector was used. The mobile phase was set at a flow-rate of 1.0 ml/min at ambient temperature. The average recovery was 68%, and the limit of detection was 0.10µg/ml. The injection volume was 20µl.

A rapid ion-pairing liquid chromatographic method was developed for the determination of penicillin G in milk described by Takeba¹⁴. Extraction was made with acetonitrile from milk and clean up solid-phase extraction with C₁₈ cartridge. Penicillin G was separated on a C-18 column

(Nucleodur, 5µm, 250x 4 mm I.D.) with a mobile phase (1ml/min) of acetonitrile-methanol-0.05 M potassium dihydrogenphosphate (20:10:80, v/v/v) mixture containing 5 mM of sodium 1-decanesulfonate adjusted to pH 3.5 and UV detection at 210 nm. The average recovery was 65%, and the limit of detection was 0.05µg/ml. The injection volume was 10µl.

The method for determination of residual sulphadimidine sodium in milk by using HPLC with a photo-diode array detector (UV detection at 266 nm) was presented, described by Furusawa⁸. A C-18 column (Nucleodur, 5µm, 250x 4 mm I.D.) and a mobile phase of 25% (v/v) ethanol solution (in water) was used. The mobile phase was set at a flow-rate of 0.8ml/min at ambient temperature. The average recovery was 93%, and the limit of detection was 0.02µg/ml. The injection volume was 10µl.

Results

We performed studies with blank and milk samples with oxytetracycline HCl, penicillin G and sulphadimidine sodium standards. The examples of typical HPLC traces for standards of blank and standard milk samples obtained under the established procedure. The resulting extracts were free from interfering compounds for detection and identification in all HPLC traces. The retention time of oxytetracycline was 6.9 min, penicillin G was 7.1 min, and sulphadimidine was 8.1 min. Oxytetracycline, penicillin G and sulphadimidine were not detected in the milk samples.

Discussion

Antibacterial drug residues have been detected in some milk samples in previous studies^{1,5,12,14,17}, but there are also some reports about no presence of these agents in milk samples^{7,8,11}. We did not detect any of the antibacterial drugs in our study. The presence of antibacterial agent residues in milk can have several drawbacks: unfavourable microbiological effects in the dairy industry, possible hypersensitivity reaction to the consumer and antibiotic resistance^{2,9,10}. The results of this study indicate that the incidence of these drugs contamination in the raw milk samples consumed in Bursa was not any risk for human health. But, there is not enough data about levels of antibacterial drugs in the bovine milks in Bursa and Turkey, so the topic needs to be more comprehensively and periodically investigated.

Acknowledgements: This research was funded by the Uludag University Research Funds (project no 99/17).

References

1. ALLARA M, IZQUIERDO P, TORRES G, RODRIGUEZ B. Penicillin G in pasteurized milk produced in Zulia State-Venezuela. *Revista Científica-Facultad De Ciencias Veterinarias* 2002;12(6):683-687.
2. CINQUINA AL, LONGO F, ANASTASI G, GIANETTI L, COZZANI R. Validation of a high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle. *J. Chromatogr. A* 2003; 987:227-233.
3. COMMISSION REGULATION (EEC): Off. J. Eur. Commun. L 102/62, 804, 1999.
4. COMMISSION OF THE EUROPEAN COMMUNITIES: Establishment by the European Community of Maximum Residue Limits (MRLs) for Residues of Veterinary Medical Products in Foodstuffs of Animal Origin, Brussels, 1991.
5. DEMET O, ACET A, TRAŞ B, BAŞ AL, EĞİLMEZ I. Konya'da faaliyet gösteren çeşitli mandralardan toplanan süt örneklerinde penisilin G, ampicilin ve penisilin V kalıntılarının araştırılması. *S.Ü. Vet. Fak. Derg.* 1992;8(1):33-35.
6. FAO/WHO: WHO Technical Rep. Ser., No. 799, 36th report, Switzerland, 1990.
7. FURUSAWA N. Rapid liquid chromatographic determination of oxytetracycline in milk. *J. Chromatogr. A* 1999; 839:247-251.
8. FURUSAWA N. Simplified determining procedure for routine residue monitoring of sulphamethazine and sulphadimethoxine in milk. *J. Chromatogr. A* 2000; 898:185-191.
9. GAUDIN V, FONTAINE J, MARIS P. Screening of penicillin residues in milk by a surface plasmon resonance-based biosensor assay : comparison of chemical and enzymatic sample pre-treatment. *Anal. Chim. Acta* 2001; 436:191-198.
10. RAMIREZ A, GUTIERREZ R, DIAZ G, GONZALEZ C, PEREZ N, VEGA S, NOA M. High-performance thin-layer chromatography-bioautography for multiple antibiotic residues in cow's milk. *J. Chromatogr. B* 2003;784:315-322.
11. SAĞMANLIGİL H, TESTERECİ H, TÜREL İ, KAHRAMAN T, ERTEKİN A. Van'da satılan sütlerde ampicilin kalıntılarının tayini ve ısıtmanın ampicilin üzerine etkisi. *Y. Y. Ü. Vet. Fak. Derg.* 1994; 5(1-2):113-120.
12. SUHREN G, HEESCHEN W. Detection of eight sulphonamides and dapsone in milk by a liquid chromatographic method. *Anal. Chim. Acta* 1993;275:329-333.
13. SUSAN E, AIELLO BS. *The Merk Vet. Manual* 8th ed. (Philadelphia) 1745-1778, 1998.
14. TAKEBA K, FUJINUMA K, MIYAZAKI T, NAKAZAWA H. Simultaneous determination of β -lactam antibiotics in milk by ion-pair liquid chromatography. *J. Chromatogr. A* 1998; 812:205-211.
15. TESTERECİ H, YÖRÜK İH, KAHRAMAN T, EKİN T, SAĞMANLIGİL H. Fluoremetric determination of ampicillin in milk with precolumn derivatization by high performance liquid chromatography. *Y. Y. Ü. Vet. Fak. Derg.* 1995; 6(1-2):105-110.
16. TÜRK GIDA KODEKSİ: Hayvansal Kökenli Gıdalarda Veteriner İlaçları Maksimum Kalıntı Limitleri Tebliği (2002/30) 3-22, 2002.
17. US FDA: National Milk Drug Residue Base, Fiscal Year 1996 Annual Report, October 1, 1995-September 30, 1996, GLH, Inc. Lighthouse Point, FL, February 10, 1997.