APPROACHES TO THE DETECTION AND CONFIRMATION OF DRUG RESIDUES IN MILK

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INTRODUCTION

The possible presence of trace residues of veterinary drugs in milk is a subject of major concern for regulatory agencies, the dairy industry, and consumers. All New Animal Drug Applications (NADAs), in addition to showing efficacy and safety of the drug to the animal, must also show that treatment of the animal will result in no hazardous residues entering the human food chain. NADAs therefore include methodology to detect tissue residues. Part of the review process at FDA's Center for Veterinary Medicine (CVM) is to evaluate the drug sponsor's residue method.

For several reasons, CVM also has its own method development program. Many veterinary drugs were approved prior to current regulatory requirements. For these older drugs, sensitive analytical methodology may not exist for residues in milk. Another problem is extra-label use of approved drugs. New drugs are approved for use in particular species for certain conditions; the label may even specify the age and sex of the animal. Very few drugs are actually approved for use in lactating dairy cattle. Careless use of drugs approved for other animals (e.g., calves), for which the milk withholding time for lactating cows is not known, may result in residues. Finally, the use of some drugs, such as chloramphenicol, has been completely banned in food producing animals. Sensitive analytical methods are needed to detect illegal use of these drugs.

For regulatory purposes, a residue method must have a high degree of both precision and specificity. In actual practice, this usually means two different types of analytical procedures, which together comprise the method. These analytical assays are referred to as determinative and confirmatory procedures.

A determinative procedure accurately quantitates residue levels in tissues. The procedure should be able to accurately determine residues at half the tolerance or concern level. For residue levels less than 0.1 ppm, the recovery from fortified tissue should be > 60% and the coefficient of variation < 20%. Interferences should be less than 10% at the residue concentration of interest. Finally, the procedure should be practicable, both in terms of time and resources needed. An experienced chemist should be able to process at least 6 to 8 (FDA will probably soon change this
number to 12) samples a day using equipment commonly available in an analytical lab.

Most chemical determinative procedures are chromatographic. A peak with a given retention time is not considered specific enough to confirm the identity of a residue. A confirmatory procedure may be a second chromatographic assay using entirely different separation conditions. However, preferred confirmatory procedures generally employ mass spectral analysis of the residue, with identification of four structurally significant fragment ions, or three ions if one is the molecular ion. FDA considers this sufficient to confirm presence of the residue. Confirmatory procedures should be able to detect residues at the concern level.

Goals of CVM method development are several-fold:

1) Lower the detection limits of existing milk methods to meet current regulatory concern levels.
2) Adapt existing tissue residue methods for use in milk.
3) Decrease reliance on organic solvents, especially halogenated solvents, in the extraction procedure. Proper disposal of hazardous waste is expensive and adds significantly to the cost of an assay. It may also result in unnecessary analyst exposure.
4) Develop multiresidue methods. Frequently, several members of a class of drugs are commercially available, and any or all of these drugs may be used in veterinary practice. Methods that detect all or most members of a drug family may be more difficult to develop, but will save time and resources in actual field use.

We briefly describe here three procedures either recently developed, or currently being developed at CVM, which illustrate these points.

LC DETERMINATIVE PROCEDURE FOR DETECTION OF TETRACYCLINES IN MILK

Introduction

Tetracyclines (TCs) are a class of antibiotics containing a partially conjugated four ring structure. Oxytetracycline (OTC), tetracycline (TC), and chlortetracycline (CTC) are available in veterinary formulations. Table 1 lists these drugs, their tolerance levels in milk as published in the Code of Federal Regulations (CFR), FDA's concern level (level at which regulatory action will be taken), and approved uses and withdrawal times in lactating dairy cattle.

In addition to these three major TCs, several other TCs are available commercially which we decided to include in this study. These are minocycline (mino), demeclocycline (DMCTC), methacycline (metha), and doxycycline (doxy). These drugs are available primarily for human use, though doxy is also approved for use in dogs.

For the purposes of this study, a target marker residue concentration of 30 ppb was selected for all seven TCs. To satisfy the above criteria for a determinative procedure, this meant that the procedure developed would need to be sensitive to at least 15 ppb. This level is that required for OTC and CTC, but is actually more sensitive than required for TC. Initial studies showed that the gradient LC conditions used by Thomas adequately resolved all seven TCs. However, interferences in the milk limited the sensitivity of the Thomas procedure to about 100 ppb.

Use of matrix solid-phase dispersion (MSPD) was also not sufficient to achieve the sensitivity we desired. At this point, we tried metal