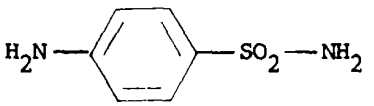
ANNEX II

GENERAL CONSIDERATIONS FOR SULFADIMIDINE AND SULFATHIAZOLE

INTRODUCTION

This Annex was prepared to discuss the many similar properties, uses, metabolism, parmacokinetics and analytical methods for Sulfadimidine and Sulfathiazole. Individual residue monographs for each drug are in this volume.

Both sulfadimidine and sulfathiazole are derivatives of p-aminobenzenesulphonamide



There are at least fifty other derivatives listed in the Merck Index and they are commonly referred to as sulfonamides or sulfas. They all exhibit antibacterial activity and many sulfonamides also are used as coccidiostats in animal production.

The two drugs are broad spectrum antibiotics which are not difficult to synthetize, thus they are not expensive to manufacture and because they are out of patent they are widely available from many manufacturing sources.

An overview would indicate that they are widely used in animal production both as therapeutic and technological drugs. If they were used as generally recommended then the residues should not be a hazard to public health, however it is well knowm that sulfonamides give rise to a large number of drug violations in farm animal products, primarily because the use often differs from that recommended. The metabolism and residue concentrations of sulfadimidine are well documented and a defined time to near zero residues or a set violative level could be established. The information on sulfathiazole is less complete. Although it is possible to set safe levels and withdrawal times based on properly carried out residue studies one should always be mindful that these drugs are often used in such a way that the residues are at higher levels than is acceptable.

Some of the residue data discussed is derived from studies where combined preparations containing either drug were used with other drugs. Most information is only available for the use of the single preparation of each drug and although it might be assumed that the concentration of residues is not very different for single or combined preparations, more caution might be needed in the case of combined preparations where the known effects of the combined preparation usually are greater than the sum of the effects of the single preparations of the constituent drugs.

USE IN FARM ANIMALS

Sulfonamides have a bacteriostatic action. They inhibit bacterial growth by preventing p-aminobenzoic acid from being incorporated into the pteroylglutamic acid (folic acid) molecule. Therapeutic preparations may contain more than one sulfonamide or be combined with other drugs; e.g. a typical preparation as used in the UK for treating enteric conditions in calves and pigs contains in a 30 ml solution: 0.6g sulfathiazole, 0.6g sulfaguanidine, 0.8g phthalysulfathiazole, 3g Kaolin, 200mg streptomycin sulphate and 67mg neomycin sulfate. Trimethoprim is often combined with a sulfonamide to produce a synergic effect which is both bactericidal and has broad spectrum activity.

Therapeutic Uses

Both drugs are highly effective against a wide range of bacterial, protozoal and rickettsial organisms. They are particularly useful in cases of septicaemia, pneumonia, enteritis and diseases of calves caused by Salmonella, Pasteurella and coliform organisms; against necrotyic enteritis in pigs and "foul in the foot" in cattle; coccidiosis in sheep and cattle and treatment - via the drinking water - of caecal and intestinal coccidiosis in poultry and rabbits; also coryza and fowl cholera. The injectable forms have also been used successfully in treating heart-water and tick-borne fever in lambs.

Use as a Technological Drug

Although sulfonamides are widely used to treat enteric conditions their use for preventing enteric diseases and in particular Atrophic Rhinitis in pigs associated with Bordatella bronchiseptica falls into the grey area of whether the compound is being used primarily as a technological drug to improve feed efficiency and liveweight gain or prophylactically as a therapeutic agent.

Sulfonamides including sulfadimidine and sulfathiazole are widely used as in-feed additives for pigs and fed to the pork and bacon pigs throughout most of their life span. The dose rate is usually about 110 g sulfonamide per kg feed. Again the commercial preparations may contain other antibiotics.

PHARMACOKINETICS OF SULFONAMIDES

The sulfonamides are organic acids which are well absorbed from the gastrointestinal tract, except the enteric compounds. They are widely distributed throughout the body and penetrate cell membranes in accordance with their degree of ionisation and lipid solubility. They are eliminated by a combination of renal excretion and biotransformation processes. Sulfonamides bind readily to blood proteins especially albumin. The fate of the sulfonamides in the kidneys involves glomerular filtration of the unbound molecules in the plasma, active carrier-mediated excretion (proximal tubule) of the ionised moiety, and reabsorption, by passive diffusion of the lipid soluble non-ionised fraction. The extent of reabsorption is determined by the pKa and lipid solubility of the sulfonamide and the pH of the tubular fluid. Urinary alkalisation decreases reabsorption by promoting ionisation within tubular fluid, and increases the solubility of the sulfonamide and its acetyl derivatives in the urine. The normal urinary reaction of herbivores is alkaline.

The pathways of biotransformation include acetylation of the aromatic amino group, which takes place in reticuloendothelial cells of the liver and other tissues, and hydroxylation of the aromatic ring which may, in turn, be conjugated with glucuronic acid. Both oxidation and conjugation are catalysed by hepatic microsomal enzymes. The N-glucose-sulfonamide and the desamino derivatives are also formed as metabolites.

Thus the major residues excreted in the urine are the parent drug, the N-acetyl and N-glucose derivatives, the desamino derivatives and water soluble conjugates.

The kinetics of excretion of sulfonamides into urine and the rate of disappearance from plasma follow first order processes. In the simplest models one equation is sufficient but other models use two competing first order equations, one for the elimination of parent drug and a second equation for the acetylation of the parent drug.

The pharmacokinetics of sulfonamides is complicated by the recycling of the drug by contact with or ingestion of contaminated bedding and housing following the excretion of drug by treated animals. This is especially a problem with pigs which are naturally coprophagic and may also happen with poultry. Where large doses are given to humans it is essential to maintain an adequate urinary flow otherwise crystalluria occurs and the drug elimination patterns do not fit the kinetic models.

RESIDUES

Introduction

There are many similarities in the metabolism and residue patterns of the two drugs and many of the analytical methods are common to both.

Because the sulfonamides have been widely used for more than forty years the information on residues is not determined by the modern and more acceptable techniques used today. One major study using C-14 radiolabelled sulfadimidine in pigs was commissioned by the Food and Drug Administration, USA (study No. 224-81-0007) and carried out in 1981. The full report of the study was published in 1984, FDA 224-81-005. This radiometric study of the residues provides the most comprehensive information on sulfonamide residues. Radiometric studies using sulfadimidine in other species or using sulfathiazole in any species have not been reported.

Metabolism

General metabolism of sulfonamides includes the acetylation of the aromatic amino group in the reticuloendothelial cells of the liver and in other tissues yields N-acetyl-derivatives, which are major metabolites of both drugs.

The desamino-sulfonamides have also been identified as residues of the parent drug in cattle (Wooley & Siegel, 1982) and may be the preferred route of metabolism in poultry (Kietzmann, 1981) but do not seem to be an important metabolic product in humans. The desamino-metabolite is thought to be formed by bacteria in the gut following oral administration of the drug. Because it is less polar than the other metabolites the desamino-metabolite is cleared more slowly and this possibly explains why desamino-sulfadimidine is present at a higher percentage of the residues as withdrawal time increases.

Small amounts (<5% of total residues) of polar metabolites are produced but have not been identified in most studies, but it is thought that they are produced by the hydroxylation of the aromatic ring which may be further conjugated with glucuronic acid.

METHODS FOR MEASURING RESIDUES

Classical method using diazotization

The aromatic amino group common to sulfonamides renders them susceptible to diazotization and coupling with specific colour reagents. The method of Bratton & Marshall, (1939), was modified by Annino (1961) and has been widely used to produce much of the data available on the residues of sulfonamides. Unfortunately the method fails to detect the Desamino-derivative because this metabolite has lost the amino group.

The sulfonamide is extracted into a protein-free solution and the drug diazotized with nitrous acid. The excess nitrous acid is destroyed with ammonium sulphamate and the diazonium salt is then coupled with N-(1-naphthyl)ethylenediamine to form a red dye. The colour is measured at 550 nm and interpolated from a standard curve prepared with pure sulfonamide.

The main metabolite of sulfadimidine and sulfatriazole is the acetyl-derivative and because the acetyl group is attached to the amine group it blocks diazotization. To overcome this the protein free extract is hydrolysed with hydrochloric acid which liberates the free amine and then the total residue of sulfonamide plus acetyl-derivative is determined. If the extract is not hydrolysed then the amount of the drug can be determined separately and the amount of residue due to acetyl-derivative can be determined by difference.

Each sulfonamide forms a different product and will not necessarily produce the same amount of colour, thus different standards should be used for specific drugs.

The sensitivity of the method is 0.050 - 0.1 ppm.

Tishler et al. (1968) also published a method based on the Bratton-Marshall reaction. Tissues and milk were extracted into chloroform/acetone and then hydrochloric acid. An aliquot off the acid extract was dried and used to develop the same diazotized products as described by Annino (see above). The method was suitable for all species of tissues and was sensitive to at least 0.1 ppm.

HPLC Methods

High performance liquid chromatography (HPLC) methods all follow the same pattern with only minor variations; - extraction of the drug into an organic phase - a clean up procedure making use of the amphoteric properties of sulfonamides, usually by dissolving or extracting the sulfonamide into strong acid or base and washing with a non-polar solvent-the pH of the extract is adjusted to 5.5-6 and the sulfonamide is extracted into a polar organic solvent. An aliquot is dissolved in a solution suitable for injection onto a C18 column and eluted with aqueous solutions of polar organic compounds (e.g. methanol or acetonitrile) and salts (e.g. acetate or phosphate).

The sensitivity of the methods varies but the most sensitive claim 0.01 ppm. For example:

Source	Drug	Tissue	Lower limit Sensitivity (ppm)
Boisseau (1988)	SMZ	Eggs, Milk	0.01
Weber & Smedley (1988)	SMZ	Milk	0.01
Lutchefeld (1976)	SMZ	Feeds	ca 0.2
Bachman et al (1976)	SMZ	Feeds	ca 0.5

Gas Cromatography (GC)

GC methods have been developed for identifying and measuring residues of sulfonamides and their metabolites. The principles of the method are the same as for HPLC except that a methylation step using diazomethane is necessary to produce the N-methyl derivative which is stable in the GC system. The method is suitable for most sulfonamides and also for the N-acetyl and desamino metabolites. (Matusik et al, (1982); Manuel & Stella, (1981), Holtmannspotter and Thier, (1982).

The lower limit of sensitivity is 0.01-0.05 ppm and is used successfully in the United Kingdom to identify sulfonamide residues.

Thin Layer Chromatography (TLC)

There are a number of TLC methods for measuring residues of sulfonamides. The sulfonamides are extracted using combinations of solvent/solvent partition especially making use of the amphoteric nature of the drugs and small column chromatography (e.g. Sep-Pak columns). The extracts are run on TLC in one or two dimensions and the spots visualised either with fluorescamine or through the Bratton-Marshall diazotization reaction and coupling with N-(1-naphtyl) ethylenediamine. The lower limit of sensitivity of the methods is ca. 0.05 ppm. A TLC method is used in many countries including Holland, Mexico and the USA, for regulating residues of sulphonamides. For TLC methods see: Haagsa et al, (1984); Torda, (1988); Sanchez, (1988); Bevill et al., (1977a); Thomas et al, (1981).

The USA is carrying out extensive tests using a TLC method with fluorescence detection to regulate sulfa residues at slaughterhouses and on the farms. The test is called the "SOS (Sulpha on Site) test" and is claimed to have helped reduce the number of violations seen with sulfa drugs. Urine is analysed by running a sample on a TLC plate and visualising the developed plate under UV-light. The test is calibrated to give a positive when the concentration of sulfonamide in urine is equivalent to a concentration in either liver or muscle <0.1 ppm. The method can be performed in a relatively short time by technicians. The results were shown to agree with those obtained by more laboratory orientated methods.

Enzyme Immunoassay

The introduction of immunoassay methods for the detection of sulfa residues is still under development. Immunoassay tests are continually coming to the market which are specific, rugged, cheap and give rapid answers. Ideally an aspecific immunoassay test is needed which will detect any one of the sulfonamides likely to be found in farm animals. Such a system is still being developed but there are available rapid enzyme immunoassay tests for sulfadimidine. Sulfadimidine can be detected in under 20 minutes in samples of urine, serum or animal feed with lower limits of sensitivity of 0.5 ppm in urine, 0.16 ppm in serum and 1.2 ppm in feed. The tests are quite simple and no specialist technical training is needed to carry them out.

Some government agencies/departments have examined these tests and found them suitable for routine screening purposes and are using them in surveillance schemes.

Mass Spectrometry (MS)

The use of MS for the unambiguous identification and quantitation of drugs is increasing. This desirable situation is well developed for sulfonamides. The UK Ministry of Agriculture has published the results of applying either GC-MS or Tandem-MS (MS-MS) to screening tissues from cattle, pigs and sheep. Fifteen compounds including sulfadimidine and sulfathiazole were known to be detectable by the GC-MS method with the lower limit of sensitivity (LLS) of 0.01 ppm. Five compounds including sulfadimidine were known to be detectable by tandem-MS with an LLS of 0.05 ppm. (UK, MAFF (1987), Food Surveillance Paper, No. 22).

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