



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

SULPHONAMIDES (2)

SUMMARY REPORT

1. The Sulphonamides play an important role as effective chemotherapeutics of bacterial and protozoal diseases in veterinary medicine. They are frequently administered in combination with dihydrofolate reductase inhibitors of the group of diamino-pyrimidines. In food producing animals, residues deplete with widely variable velocity depending on many factors such as the nature of the compound, its formulation and route of administration, the treated animal species, genotypes etc.
2. The Sulphonamide group includes a large number of very old compounds and adequate toxicological data meeting modern requirements for testing, together with carcinogenicity studies and mutagenicity data, are not available for the majority of these compounds.
3. From the overall picture, however, it is clear that the number of effects which are relevant for the assessment of low level exposure to residues are limited. Potential effects, which are not always related to dose, and which may be limited to predisposed humans, include allergic reactions.
4. Several Member States have previously adopted tolerances of 100 µg/kg, based on old data, as had certain third countries, and this tolerance was considered to provide a sufficient margin of safety.
5. In the case of sulfamethazine, however, additional studies have been undertaken, including recent carcinogenicity studies. Sulfamethazine has recently been evaluated by the 34th Joint WHO/FAO Expert Committee on Food Additives which concluded that the thyroid tumours in rodents were most likely due to hormonal disturbance and that humans exposed to sulfamethazine below a threshold level would not be at carcinogenic risk. The JECFA also established a maximum residue limit of 100 µg/kg for sulfamethazine in meat.
6. In these circumstances the Committee for Veterinary Medicinal Products considers that a tissue MRL of 100 µg/kg of the original drug substance should be applied to all compounds of the sulphonamide group.

Considering:

- Σ the available toxicological data, which suggest that the metabolites of the sulphonamides are within the same range of toxicity as the parent compounds;
- Σ the available pharmacokinetics studies;
- Σ the need to provide for simple analytical detection methods whenever possible.

the Committee does not consider it necessary to recommend the inclusion of any of the metabolites within this tolerance at present.

7. Residues of sulphonamides in tissues can be routinely monitored at or below the above required limits using, for example, High Performance Liquid Chromatography.

Reliable confirmatory or reference methods are based on known procedures using gas chromatography/mass spectrometry.

8. In addition, there are several validated routine analytical methods for the determination of sulphonamides in milk, based on HPLC assays. The methods are specific for each sulphonamide and have limits of quantification of at least 50% of the current provisional MRL of insert 100 µg/kg. The marker residue is the parent drug. It is recommended that the current MRL for milk of 100 µg/kg be adopted for the sulphonamide group. There is sufficient published evidence to demonstrate that the pharmacokinetics, including metabolism and excretion in milk, of sulphonamides is similar in sheep and goats to those of cattle, and so the MRL also applies to those species.
9. The Committee for Veterinary Medicinal Products recommends that the sulphonamides are entered into Annex I of Council Regulation (EEC) No 2377/90 as indicated in the following table:

Sulphonamides

Pharmacologically active substance	Marker residue	Animal Species	MRLs	Target tissues	Other provisions
Sulphonamides	Parent drug	Bovine Ovine Caprine	100 µg/kg	Milk	The combined residues of all substances in the sulphonamide group should not exceed 100 µg/kg

Confirmatory methods of analysis of sulphonamides in tissues and milk are already available for use in Member States. However, a general method of analysis covering both meat and milk is attached.



A critical review of methods for the control of sulphonamide residues in animal tissues and body fluids

Many approaches for the determination of sulphonamides in tissues of food-producing animals have been developed utilising chemical, microbiological or immunological methodologies. Reviews of these approaches are published by Agarwal and Guggisberg et al. (1,2).

Microbiological methods are easy to perform and allow a high sample throughput, but they lack both sensitivity and specificity.

Immunological approaches may be sensitive enough to satisfy the MRL for sulfonamides of 100 µg/kg and allow also a high sample throughput, but they lack the necessary specificity. Both microbiological and immunological methods provide a good tool for screening analyses.

Chemical methods for the determination of sulphonamides are available utilising GC and HPLC with various types of detection. Among these methods HPLC provides a very effective tool since the sample throughput is relatively high and the specificity is satisfying when using appropriate detection methods like UV-, electrochemical or diode array detection (1). The review of the HPLC methods described in the literature leads to the conclusion, that in most cases the clean-up method will determine whether a given approach is useful in routine application or not.

To our knowledge, most of the published and reviewed methods mentioned above are not validated according to international standards. An exception, however, is a robust multi-method which was published by Malisch et al. (3,4,5). This method meets the high quality criteria of the Commission Decision 93/256/EEC, originally set up for the residue control of hormones and thyreostatics, as well as the criteria required in the framework of Directive 81/852/EEC, which are described in volume VI of the Rules Governing Medicinal Products in the European Community (6). Therefore, only this method will be dealt with in this review.

The method was developed for the residue analysis of sulphonamides and other chemotherapeutics in meat, milk and eggs. The principle of the method is given in the flow chart below and comprises the extraction of the drug residue into buffer and acetonitrile, filtration, separation of the aqueous phase, removal of fat and disturbing polar substances. After this rather time-consuming sample processing, the analysis is finally achieved utilising HPLC with photodiode array detection.

Short description of the method (3,4,5):

15 g homogenised meat sample is extracted with a mixture of a citrate/phosphate buffer and acetonitrile. After filtration, an aliquot corresponding to 10 g is transferred into a separating funnel. Water is separated after addition of salt and butyl methyl ether/hexane. The organic layer is dried with sodium sulfate and concentrated after addition of ethylene glycol as a "keeper" (keeps substances in solution).

To remove lipid co-extractives, the residue is rinsed with small volumes of hexane, acetonitrile and buffer into a screw cap test tube. After shaking, the upper hexane layer is taken off and rejected. To remove polar disturbing substances, a small volume of water is added and then the lower layer is extracted with ethyl acetate twice. After addition of ethylene glycol, the combined ethyl acetate phases are evaporated to "ethylene glycol dryness". The final volume is 1.0 ml (ethylene glycol/water [1/1]). The evaporation processes can be fully automated with the help of a centrifugal vacuum concentrator or a rotary evaporator.

Sulfonamides and, if necessary, also their acetyl metabolites are determined by reverse phase high performance liquid chromatography (HPLC) with diode array detection.

Performance parameters of the method:

Specificity, interference:

Nicotinamide and, occasionally, caffeine can cause interferences. Since the method utilises spectrometry, the comparison of UV spectra and co-chromatography are mandatory when confirming the results.

Limit of detection and limit of determination:

Depending on the substance and the wave length used, the limit of detection varies between 4.6 and 37.6 µg/kg; whereas the limit of determination varies between 8.1 and 68.3 µg/kg.

For sulphanilamide, however, the limit of detection varies between 17.9 and 67.6 µg/kg; the limit of determination varies between 28.7 and 113.2 µg/kg respectively, depending on the detector.

Recovery, linearity and repeatability

Recovery tests with spiked samples confirmed the linearity of the method in a large range. The overall recovery varied between 80 and 110% in the spike range of 50-10,000 µg/kg (sulfathiazol and sulfonilamide provide 80-95 %). The coefficient of variation for repeated analysis did not exceed 15% and thus met the requirements of the EU.

Own experiences with the method:

Though laborious and rather time-consuming, the method has been successfully applied in our laboratory.

The method is very suitable for the analysis of muscle, but not for liver or kidney due to the considerable interferences of these matrices.

Another disadvantage of the method is the rather low sample throughput due to its complexity.

Conclusions:

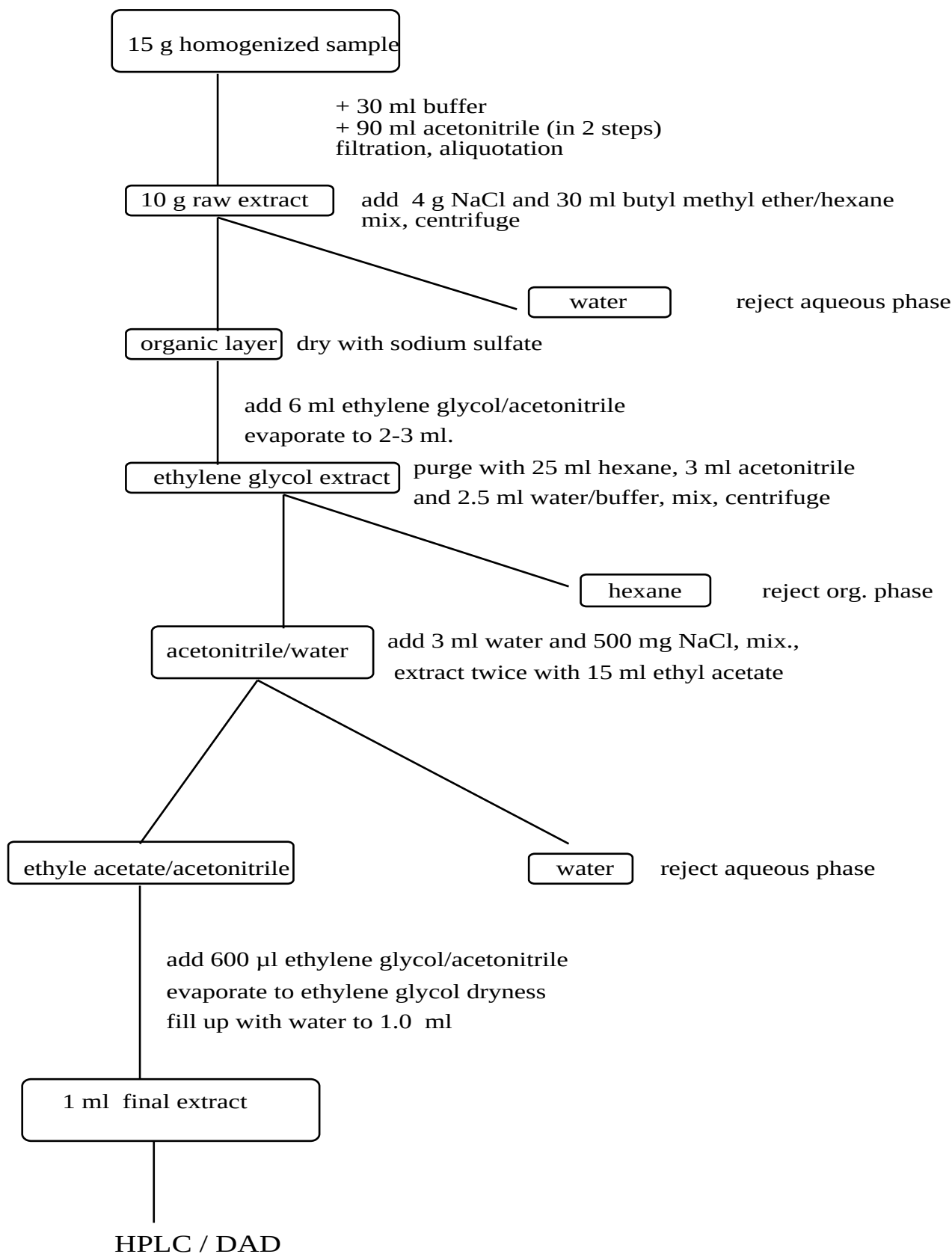
A versatile HPLC method is available for the determination of residues of sulfonamides in muscle, which meets the requirements of Vol. VI of The Rules Governing Medicinal Products in the European Community.

Efforts should be made to simplify and to enhance the clean-up procedure in view of their application to other matrices like liver and kidney.

Moreover, efforts should be made to find an appropriate internal standard which might increase the reliability of the quantitative results.

References:

1. Agarwal, V.: High-performance liquid chromatographic methods for the determination of sulphonamides in tissue, milk and eggs. *J. Chromatogr.* **624** (1992) 411-423
2. Guggisberg, D; Mooser, A.E.; Koch, H.: Methods for the determination of sulphonamides in meat. *J. Chromatogr.* **624** (1992) 425-437
3. Malisch, R.; Bourgois, B.; Lippolt, R.: Multiresidue analysis of selected chemotherapeutics and antiparasitics. Part I. Determination of sulphonamides and their N4-metabolites, chloramphenicol and nicarbazin in meat. *Dtsch. Lebensm. Rdsch.* **88** (1992) 205-216
4. Malisch, R.: Multiresidue analysis of selected chemotherapeutics and antiparasitics. Determination of sulphonamides and their N4-metabolites, chloramphenicol and nicarbazin in meat. Documentation for the Codex Committee Residues of Veterinary Drugs in Food, Ad Hoc Working Group, Methods of Analysis and Sampling, Eighth Session, Washington, D.C., 7-10 June 1994.
5. Malisch, R.: Nachweismethoden für Rückstände von Arzneimitteln: Vorläufige Methode zur Bestimmung und zum Nachweis von Sulfonamiden in Muskelfleisch - HPLC-Verfahren. *Bundesgesundhbl.* 6/94, p. 279-282
6. The Rules Governing Medicinal Products in the European Community, Vol. VI ISBN 92-826-3173-7, ECSC-EEC-EAEC, Brussels, Luxembourg, 1991



Flow chart of the sample clean-up according to Malisch