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THE USE OF MICROTRACERS® IN A MEDICATED PREMIX TO

DETERMINE THE PRESENCE OF TIAMULIN IN FINAL FEED

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ABSTRACT

Microtracers® consisting of iron particles coloured with codified food dyes (Micro-Tracers Inc., San Francisco) were incorporated into a tiamulin (2%) premix. On dilution, to give drug concentrations of 100, 30 and 10 ppm, feed was assayed for both drug and microtracer. Good correlation was found between drug and microtracer recoveries. Thus the use of microtracers gives an easy and rapid method for semi quantitative detection of tiamulin in a non laboratory i.e. field environment.

INTRODUCTION

Diseases in animals are often treated, not by the administration of the drug in a conventional unit dosage form, but rather by the addition of the drug to the animal feed (1). Thus the treatment of choice for swine dysentery is tiamulin (Tiamutin ® - Sandoz Ltd.); the medication is often administered in the feed over an intensive period of 1 to 8 weeks, at concentrations of from 30 to 100 ppm. The drug is supplied to the miller/veterinary surgeon as a Medicated Premix containing 2% drug and requiring dilution with feed to the concentration level appropriate to the prescribed treatment regimen (2).

Consequently it may be necessary at farm level to monitor the animal feed for the presence of the antibiotic or other additive residues which may appear due to carry-over in mixing, handling or storage of ingredients. Such quality assurance tests may be undertaken to provide either qualitative or quantitative information on the drug in question, the former to indicate the presence of the actual drug, the latter to indicate whether it is present at the required concentration. Such tests could be particularly important in the case of feeds containing tiamulin as this drug shows dose-related incompatibility with a number of ionophore feed additive coccidiostats such as monensin, narasacin and salinomycin.

Conventional analytical techniques usually require the services of specialist laboratories and the time lag between submission of samples and receipt of results usually prevents remedial action. Often feed analysis serves as a basis for establishing claims for compensation, rather than contributing to favourable performance. Micro-Tracers, Inc. of San Francisco, California, has developed a unique tracer system comprising dye impregnated particles of either graphite or iron. By incorporating the tracer into micronutrients or their premixes in a fixed ratio, rapid analysis with simple equipment can be carried out, to provide instantaneous qualitative and quantitative analysis. The principal value of the tests is that they can be performed with an acceptable degree of accuracy by relatively unskilled personnel, under field or office conditions (3).

The objectives of the current project were therefore to 1) assess the suitability of Microtracers as a rapid indicator of the presence of Tiamulin in animal feed; 2) determine if the inclusion of Microtracers at a level of 3g per Kg. in Tiamutin Premix 2% could distinguish - 3) between final feed drug levels of 100 ppm 30 ppm and 10 ppm compare recoveries of drug and Micro-Tracers from feed and a pelleted feed product at 100 ppm.

In veterinary practice, it is not only important to determine the presence or absence of tiamulin but also to be able to distinguish between high and low dose feeds because of the potential for drug interactions with the ionophore coccidiostats.

MATERIALS and METHODS

Microtracer {FS. Violet. (Red #3 - Blue#2)} was added to Tiamutin Premix at Leo Laboratories and taken to Luceys Millers Ltd., Ballymakeera, Co. Cork for addition to feed to contain final feed tiamulin concentrations of 10, 30, and 100 ppm. 3 gm Microtracer FS Violet (Red # 3 - Blue # 2) were used per Kilo of medicant . (Tiamutin 2%).

Microtracer Assay - Tiamulin In Pig Feed

Three different levels of medication were used in the trials, and this was accomplished in 1.5 tonne batches . Multiple samples were withdrawn as each batch exited from mixer. It was suggested that in making the Microtracer counts 250 gms, 75 gm and 25 gm of finished feed respectively should be used to give satisfactory numbers of tracers per sample in 10, 30 and 100 ppm timeline finished feed. Pelleted feed samples were also counted. The pellets when ground (coffee grinder), gave mean count values approximately 70% of those obtained from the unpelleted feed. This is acceptable bearing in mind that the feed contained relatively high fat and oil content levels and was pelleted as pencils (4). Samples were analysed using the portable Microtracer rotary detector , based on magnetic collection of Microtracers, and the counts obtained were recorded.

Results were calculated on an IBM/PC using the Microtracer "Mixer" programme (5). Initial microtracer counts on the Tiamutin Premix used in these trials showed that approx 3gm of Microtracer FS Violet had been added to each kilo. This test was carried out on site at the mill using portable equipment. The calculation was done by analyzing 30 gm of sample.

Chemical Assay of Tiamulin in Pig Feed:

Samples (n=6) from each feed (i.e. 10, 30 and 100ppm feeds and 100ppm pelleted feed) were assayed by HPLC.

Samples were extracted in the presence of sodium carbonate solution(1% w/v) with a mixture of hexane and ethyl acetate (75:25 v/v). The tiamulin base present in the organic phase was then extracted with a tartaric acid solution (0.1% w/v) , which was applied onto an HPLC column using a sample loop. The extinction of the analysis solution was measured at 250 nm and the content of tiamutin was calculated with the aid of a calibration graph.

The Chromatographic Conditions employed were: column: Technopak 10 C18 25cm, Mobile Phase: 70:35:45 methanol: acetonitrile: 1% ammonium carbonate, Flow Rate: 1.5 ml min⁻¹, UV Detection@ 250 run, Loop Volume: 200ul.

RESULTS & DISCUSSION

The feed sample sizes taken 250gm, 75gm, and 25gm were chosen so as to produce an expected Microtracer count of 15 particles per sample. These Microtracer values were converted to the corresponding concentration estimates, i.e. "ppm" (count). Six samples of each feed and of the pellets were randomly chosen for chemical assay of tiamulin.

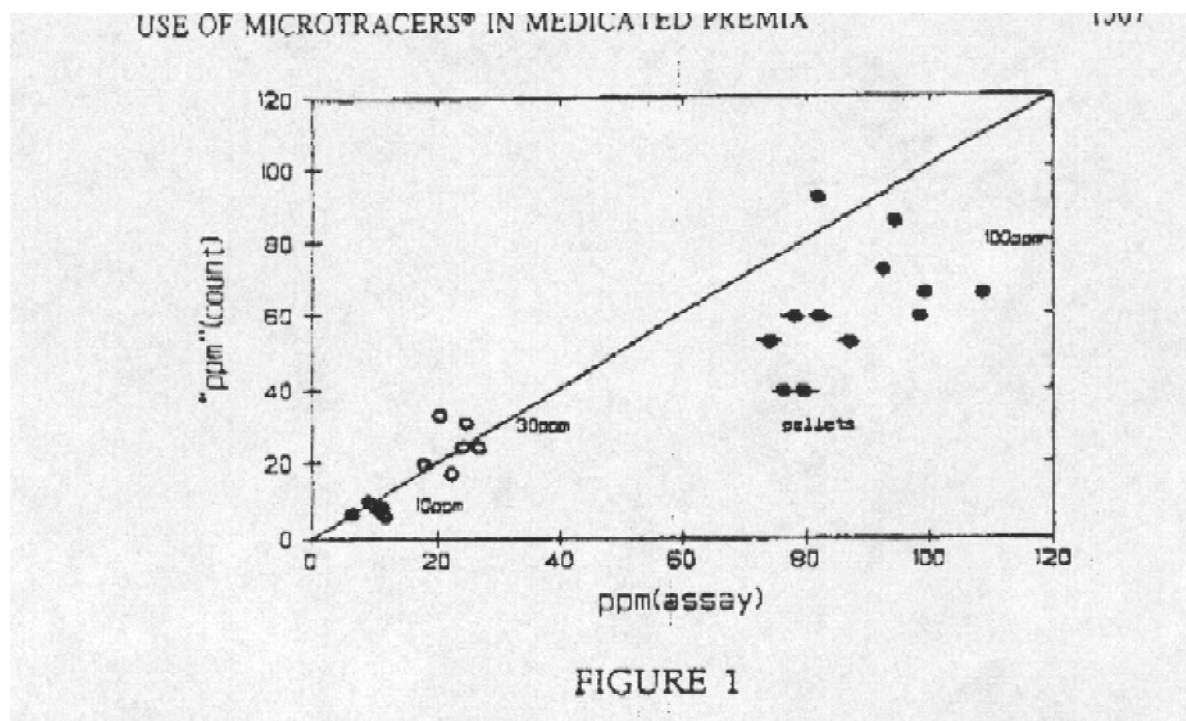


FIGURE I

Comparison of individual assay values of the Tiamulin content of pig feed sampler, obtained using the Microtracer assay ("ppm" (count)) and the HPLC assay ("ppm," (assay))

hydrogen Fumarate by HPLC. Mean HPLC Tiamulin assay results, corrected for degradation were in good agreement with expected values for the 10ppm and 100ppm feed mixes. (Table 1). Both the 30ppm feed mix and the pelleted 100ppm feed mix samples gave lower values than expected (75 to 80%). Pelleting is associated with increased drug degradation when stored over prolonged periods e.g. several months.

The Microtracer "assay estimates" ("ppm" (count)) are plotted versus the HPLC Tiamulin assay results in Figure 1. Also included is the "perfect" correlation line. The data is scattered around the line for the 10 ppm and 30ppm samples. The 100 ppm samples and particularly the pelleted samples tend to fall below the line. This trend indicates incomplete Microtracer particle recovery and is not surprising in the case of pelleted samples. The mean data are summarized in Table I and plotted in Figure 2.

TABLE 1

Tiamulin Content of Pig Feed and Pellet Samples

Expected ppm	Assay Value (ppm assay)			Microtracer ("ppm" count)		
	\bar{x}	S.D.	C.V.	\bar{x}	S.D.	C.V.
10 feed	9.8	1.66	17%	7.26	1.53	20%
30 feed	22.6	2.72	12%	24.7	5.96	24%
100 feed	96.0	7.33	7.6%	73.1	12.6	17%
100 pellets	79.4	3.89	4.9%	50.1	8.93	18%

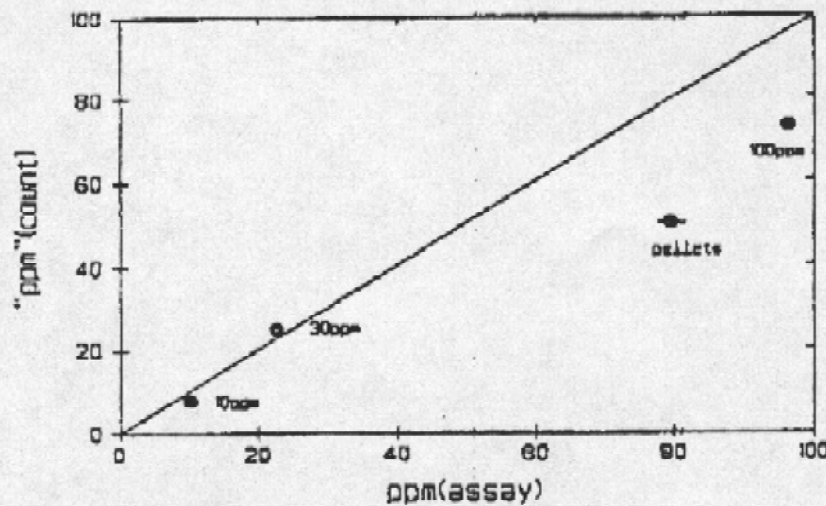


FIGURE 2

Comparison of the mean (n=6) ppm estimates of the Tiamulin content of pig feeds obtained using the Microtracer ("ppm"

(count)) and the HPLC (ppm (assay)) methods.

The Microtracer results show that the samples are adequately mixed as the tracer counts conform to what would be expected from a Poisson Distribution. While there is greater variability in the estimate of drug content using Microtracer, the results can be considered to reflect the content of drug in the feed.

The standard deviation of the Microtracer results demonstrates that the Microtracer technique employing counts on six samples should readily distinguish feeds of 100ppm from those of 30ppm and 10ppm, tiamulin. Furthermore feeds containing 30ppm and 10ppm. tiamulin should also be distinguishable. Thus the use of microtracers gives an easy and rapid method for semi quantitative detection of tiamulin in a non laboratory i.e. field environment, despite the large differences in density between microtracer and drug.

REFERENCES

1. Nessel R.J., in *Formulation of Veterinary Dosage Forms* ed J. Blodinger, p 245 Pub; Marcel Dekker Inc., N.Y., 1983.
2. O' Loan A., Ryan J., Wilkinson M.L., in *Tiamutin Biochemie GmbH, Kundl, Austria.*
3. Shane, S.M., *Feed Management* 57, Dec, (1982)
4. *Quality Assurance with Microtracers F.S., Item FF .Micro Tracers, Inc. May, 1985.*
5. *Mixer- Chi-Square Distribution Programme, Micro Tracers, Inc. IBM.*