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Dissolution test for ivermectin in oral veterinary paste

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A dissolution test for oral veterinary pastes with ivermectin using the Ph. Eur. paddle apparatus was developed. Sink conditions were achieved with sodium lauryl sulphate in a concentration of 0.5% as dissolution medium. By means of HPLC fast degradation of ivermectin was observed in HCl 0.1 M solution. Rotation speed of the paddle at 75 rpm was appropriate as demonstrated in a study comparing two different products.

Ivermectin, an anthelmintic drug, is a macrocyclic lactone used in pharmaceutical products as a mixture of two components: $H2B_{1a}$ and $H2B_{1b}$. Ivermectin is practically insoluble in water, freely soluble in methylene chloride and soluble in alcohol (Ph.Eur.). The drug exhibits microfilaricidal action in onchocerciasis and is also active in some other worm infections. It is used in human and in veterinary medicine. The drug is absorbed from the gastrointestinal tract with peak plasma concentrations after approximately 4 h. It can be administered orally as tablets, but the most popular formulation in veterinary is an oral semisolid suspension. The aim of the study was to develop an *in vitro* dissolution test for oral veterinary pastes containing ivermectin, intended for horse treatment. Since the drug is active only when released from the formulation, the test is useful as a tool for quality control and in the product development process (FIP 1997; Siewert et al. 2003). Two products from different manufacturers (A and B) were used in the study.

A dissolution test for semisolid oral preparations is not described in pharmacopoeias or other official documents. The investigated pastes can be considered as suspensions of high viscosity and semisolid consistency. Thus methods used either for oral suspensions or topical semisolids can be taken into consideration (FDA 1997; FIP 1997; Siewert et al. 2003). A Ph.Eur. paddle dissolution apparatus was employed in our studies. Samples containing 40 mg of ivermectin were used, which corresponds to 4 g and 2 g of the paste A and B, respectively (the dose of ivermectin for horses is 200 μg/kg; for example a single dose container is filled with 15 g of the 2% paste).

The choice of the dissolution medium is based on solubility of the active substance and requires addition of surfactants or organic solvents when the substance is insoluble in water in order to maintain "sink conditions" (FIP 1997). The solubility of ivermectin in common dissolution media was determined and is presented in Table 1. The results show that water and phosphate buffer do not offer sink conditions when used at a volume of 900 ml. This was confirmed in a dissolution study – after 30 min only 12–15% of the drug was released into a phosphate buffer and no further release was observed over the next 3 h.

Increased solubility was achieved in sodium lauryl sulfate (SLS) solutions. Low concentration of SLS, namely 0.5% (w/w), can be used to provide sink conditions for ivermectin if the paste samples are between 10–20 g. Dissolution profiles in pH 6.8 buffer containing 0.5% SLS and in aqueous SLS solution were similar (data not shown), indicating that there is no need to use buffered solutions.

Ivermectin undergoes fast degradation in an acidic solution (Table 2) and the process is accelerated in the presence of surfactant (Table 1). The main products of degradation appeared as additional peaks on HPLC chromatograms with relative retention times of 0.45 and 0.58 min. For this reason HCl cannot be used as dissolution medium. In addition a two stage test with a change of media is not possible, although such a test could be reasonable since the gut is the site of action of the drug.

On the basis of the preliminary studies, 0.5% SLS solution (900 ml) was used as dissolution medium.

The test was performed for 13 samples of the product A, whose mass was in the range 3.7–6.1 g. The weight of the samples did not influence the results. The reproducibility of the dissolution profiles was not very good, probably because of the semisolid type of the suspension: after 60 min 92.2% of the drug was released and the relative standard deviation was 11.3%. For two samples the amount of the drug released after 60 min to SLS solution was below 80%.

In a separate experiment a new batch of product A was compared with product B. The amount of ivermectin released after 60 min from paste A was $85.0 \pm 8.7\%$ and from paste B $69.6 \pm 10.8\%$. After 120 min the doses released were $104.4 \pm 4.4\%$ and $86.6 \pm 10.8\%$ for paste A and B, respectively. The difference between the two products was statistically significant (ANOVA, $p < 0.05$). The observed lower release rate of ivermectin from product B is related to the character of the base – the base in the paste A completely dissolves in the acceptor fluid within 1 h, while paste B disintegrates slowely during at least 3 h.

Table 1: Solubility of ivermectin in various dissolution media (37 °C)

Solvent	Solubility (mg/ml)
Water	4×10^{-3}
HCl 0,1 M	degradation
Phosphate buffer pH 6,8	12×10^{-3}
2% (w/w) SLS	more than 6.0
1% (w/w) SLS	4.54
0.5% (w/w) SLS	2.80
0.5% SLS in HCl (0,1 M)	degradation; yellow color is developed after 4 h

Table 2: Degradation of ivermectin in HCl 0.1 M solution

Time (h)	% of the initial content	
	20 °C	37 °C
1	not studied	59.5
2	69.1	46.2
6	38.1	9.9

According to recent recommendations, the rotation speed of the paddle during the test for oral suspensions should be 25 rpm, increasing up to 50 or 75 rpm for high viscosity products (Siewert et al. 2003). When the agitation rate was reduced to 50 rpm this had no major effect on the release rate from paste B, but influenced the dissolution profile of product A significantly. In addition the reproducibility of the results diminished. For example the amount of ivermectin released from paste A after 30 min was 42.0 ± 29. 9% in comparison to 61.5 ± 9.9% at the higher agitation. Thus rotation speed of 75 rpm should be considered appropriate and should not be increased if the distinguishing power of the test between different preparations is to be achieved (Siewert et al. 2003).

Experimental

1. Materials

Oral pastes for horses from two different manufacturers were used containing 10 mg/g (A) or 20 mg/g (B) of ivermectin, respectively. The quantitative assay for ivermectin was performed and the content was 104.4–107.7% of the declared concentration.

2. Dissolution test

The test was performed with a Ph.Eur. paddle apparatus (Pharma Test, Heinburg, Germany), at agitations of 75 rpm or 50 rpm, at 37 ± 0.5 °C. Sodium lauryl sulphate (BDH Chemicals, Poole, England) 0.5% (w/v) aqueous solution was used as a dissolution medium (900 ml). The paste (4 g of A or 2 g B) was placed into the vessel with a syringe with canule (A) or dropping the sample at the surface of the dissolution medium (B). After the time intervals (15, 30, 45, 60 min and 2 h), a sample of the dissolution medium was withdrawn (2 ml), filtered through a glass filter and analysed.

3. HPLC analysis

The determination of ivermectin was performed with a modified HPLC method as described in Ph.Eur. The analysed solutions were diluted with methanol (1 : 10). The HPLC system consisted of octadecyl column (250 × 4 mm; 5 μm; Lichrospher RP-18; Merck), an integrator D-12500 A, a detector UV-Vis L-4250, pump L-6200A (Merck-Hitachi, Darmstadt, Germany). A mixture of acetonitrile : methanol : water (64 : 24 : 16) was used as the mobile phase with a flow rate 1.9 ml/min. The volume of the injected sample was 20 μl and detection was performed at 254 nm.

4. Solubility studies

An access of the substance was shaken in tightly closed glass flasks with a suitable solvent (Table 1) for 20 h at 37 ± 0.1 °C. The suspensions were filtered and the filtrate, after dilution in methanol, was analysed by HPLC.

5. Stability in acidic solution

Solutions of ivermectin in HCl 0.1 M were stored in tightly closed glass vials at 20 °C and 37 °C. Chromatograms of the solutions were recorded at t = 0 and after 1, 2 and 6 h. The area of the ivermectin (retention time 10.5 min) and additional peaks were calculated.

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Chemical properties of a mannoglucan from *Cistanche deserticola*

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A new mannoglucan from *Cistanche deserticola* from China is characterized. The compound is responsible for a mild stimulation of mitogen-induced T and B lymphocyte proliferation.

Cistanche deserticola Y. C. Ma is a holoparasite growing on the roots of the dicotyledonous plant *Haloxylon ammodendron* Bunge which is widely distributed in the northwest of China. As it is an important tonic in oriental medicine, many active components and pharmacological activities have been claimed (Karasawa et al. 1986; Yoshizawa et al. 1990). These properties might be associated with some of the polysaccharide components of *Cistanche* which have not yet been investigated. Mitogenic pectic polysaccharides and neutral polysaccharides have been isolated and characterized from *Cistanche deserticola* from Mongolia (Radna et al. 1996; Anna et al. 1997; Bozena et al. 1999). Here we report the chemical properties of a new mannoglucan from *Cistanche deserticola* from China, which is responsible for mild stimulation of mitogen-induced T and B lymphocyte proliferation.

After the stems of *Cistanche deserticola* Y. C. Ma were extracted with 95% ethanol, cold distilled water was used to extract the residue. The cold water extract was precipitated with EtOH, protein was removed and dialyzed, obtaining the crude polysaccharide CCDP. The crude polysaccharide was fractionated on a DEAE column and a Sephadex G-150 column to afford a protein-free polysaccharide (CDP-6), which comprised glucose (89.2%) and mannose (10.4%), and of which the average M_r was estimated to be 6.8×10^4. The polysaccharide CDP-6 showed $[\alpha]_D^{20} + 42^\circ$ (c 1, H_2O). The absolute configurations of the sugars were determined by GC of TMSi (−)-2-butylglycosides. CDP-6 was methylated (Needs et al. 1993), hydrolyzed, converted to the alditol acetates, and analyzed by

Table: Methylation analysis of CDP-6 mannoglucan

Methylated alditol acetate derivatives	Molar ratio[a] CDP-6	CDP-6-1	CDP-6-12
2,3,4-tri-OMe Glc	22.8	20.1	4.1
2,3,4,6-di-OMe Glc	0.3	3.7	1.0
2,4-di-OMe Man	2.3	1.2	0.8
2,3,4-di-OMe Man		0.9	

[a]Calculated from peak areas and molecular weight of derivatives