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SHORT COMMUNICATION

Twenty-Week Solid-State Stability Study of Combined Doxycycline, Trimethoprim and Sulfamethoxypyridazine Formulations after Extemporaneous Preparation for Veterinary Use

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A B S T R A C T

The solid-state stability of doxycycline, trimethoprim and sulfamethoxypyridazine alone or in combination was evaluated for 135 days (20 weeks). A validated HPLC assay was developed for their simultaneous determination and applied to assess their solid-state stability alone and in binary combinations upon extemporaneous preparation. The results showed that the drugs are stable based on ICH Q1A (R2) protocol for at least 135 days at 25±2ºC and at 60±5% of relative humidity and protected from light. Similarly, the solid-state stability of the combination mixtures of doxycycline and sulfamethoxypyridazine, doxycycline and trimethoprim, and trimethoprim and sulfamethoxypyridazine were also shown stable in the above conditions. These findings provide evidence for the suitability of preparation of extemporaneous formulations which might help for safe and efficacious treatments in veterinary medicine.

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INTRODUCTION

Combined formulations of various antibiotics are common to increase the efficacy against emerging resistance and enhance the antibiotic's spectrum of activity in veterinary medicine (Poole and Sheffield, 2013; European Food Safety Authority, 2013). Formulations that contain sulfonamides in combination with other active drugs like doxycycline are particularly relevant to treat gram-positive and gram-negative bacteria. Doxycycline, has demonstrated activity against some anaerobes microorganism while sulfamethoxypyridazine is considered ineffective against most obligate anaerobes (Langston, 2003). Other combinations used in veterinary medicine include trimethoprim with sulfamethoxy- pyridazine or in combination with doxycycline or other tetracyclines antibiotics (Hughes *et al*., 2013).

These combined formulations may be prepared extemporaneous before administration to farmed animals or prepared in larger amounts and stored appropriately until administration, provided that the active components remain unchanged and without degradation (Langston, 2003; Papich *et al*., 2013). Stability testing may provide evidence to assess the quality of a drug substance, the product variations over time and the influence of environmental factors such as temperature and humidity. This information enables recommendations for storage conditions, re-testing schedules and the shelf life parameters to ensure the quality of the product remains unaltered. The stability of doxycycline and other tetracyclines has been studied in animal feed and water, in a variety of formulations including suspensions, slow release systems, tablets, capsules (Injac *et al*., 2007) as well as extemporaneous preparations (Papich *et al*., 2013). Similarly, the stability of trimethoprim alone or in combination with sulfonamides (sulfamethoxazole) has also been evaluated (Bettinetti *et al*., 2000). The study of the stability of combination formulation is particularly relevant to ensure correct dosage and greater rational use (Paphitou, 2013) beneficial to protect the food chain, the environment, overall public health and to address emerging antibiotic resistance (Saqib *et al*., 2012).

MATERIALS AND METHODS

The solid-sate stability of doxycycline hyclate, sulfamethoxypyridazine sodium and trimethoprim glutamate (Polichem SA, Reus, Spain) alone or in binary

combinations (Table 1) were studied under controlled conditions of temperature 25±2ºC and 60±5% relative humidity following the ICH Q1A(R2) protocol for stability testing of new drug substances of the European Medicines Agency. Each drug or their study combinations were stored under these conditions and samples (n=3) collected at 0 (control), 15, 30, 45, 60, 75, 90, 105, 120 and 135 days and the drug amount analyzed by HPLC (Kontron Instruments, Barcelona, Spain). A validated method was adapted from the literature and developed for the simultaneous determination of doxycycline, sulfamethoxypyridazine and trimethoprim in the different study formulations (Injac *et al*., 2007). Samples (20 µl) were injected in a Nucleosil C_{18}, (5 µm, 120×4.0 mm) column (Teknokroma, Barcelona, Spain) at room temperature and 1 ml/min flow rate and eluted with two different mobile phase conditions: Mobile phase in elution conditions I was a 31/69 (v/v) mixture of acetonitrile (HPLC grade) and 0.1M, pH=2.75 acetic acid and the UV detector set at 230 nm. In elution conditions II, the mobile phase was 35/65 (v/v) mixture of acetonitrile (HPLC grade) and pH=2.5 buffer mixture (0.1M 1-hydrate citric acid and 0.2M di-sodium hydrogen phosphate anhydrous) and detection set at 350 nm (Table 1). Standard stock solutions of doxycycline, trimethoprim and sulfamethoxypyridazine were prepared in methanol (HPLC grade) at 2.4, 0.6 and 16 mg/mL concentration respectively, stored at -25±2ºC and used daily to prepare quality control samples and the external calibration curves which ranged 15-120 µg/ml, 5-30 µg/ml and 50-400 µg/ml respectively. The methods developed for both elution conditions were linear within the target concentration range (r^2 >0.990), the lower limits of quantification were 15.1±0.49, 50.4±1.1 and 4.98±0.06 µg/ml for doxycycline, sulfamethoxypyridazine and trimethoprim respectively. The intra-day, inter-day precision and accuracy were within 15% deviation and recovery was above 70% (Table 2). Under these conditions, doxycycline eluted at 2.2 min, sulfamethoxypyridazine at 1.8 min and trimethoprim at 1.9 min without any matrix or drug interferences. Lastly, elution conditions II was chosen to quantify sulfamethoxypyridazine due to better chromatographic resolution.

RESULTS AND DISCUSSION

The degradation profiles for each drug or their binary combinations are shown in Fig 1. Doxycycline, sulfamethoxypyridazine and trimethoprim alone or in their binary combinations were stable at 25±2ºC, 60±5% of relative humidity, protected from light, and no degradation over 135 days was observed. The amounts of drug remained within 95-105% of label record amount and within the accepted shelf-life required parameters of 90-110% range. In addition, an attempt to identify a model describing the degradation kinetic (zero-order or first-order) was also done. However, the lack of degradation did not allow the identification of the slope to proceed fitting in any of the drugs tested. Based on this stability result, the binary combinations of these drugs met the required solid-state stability and are suitable for extemporaneous preparation of specific veterinary treatments in accordance with regulatory requirements (European Food Safety Authority,

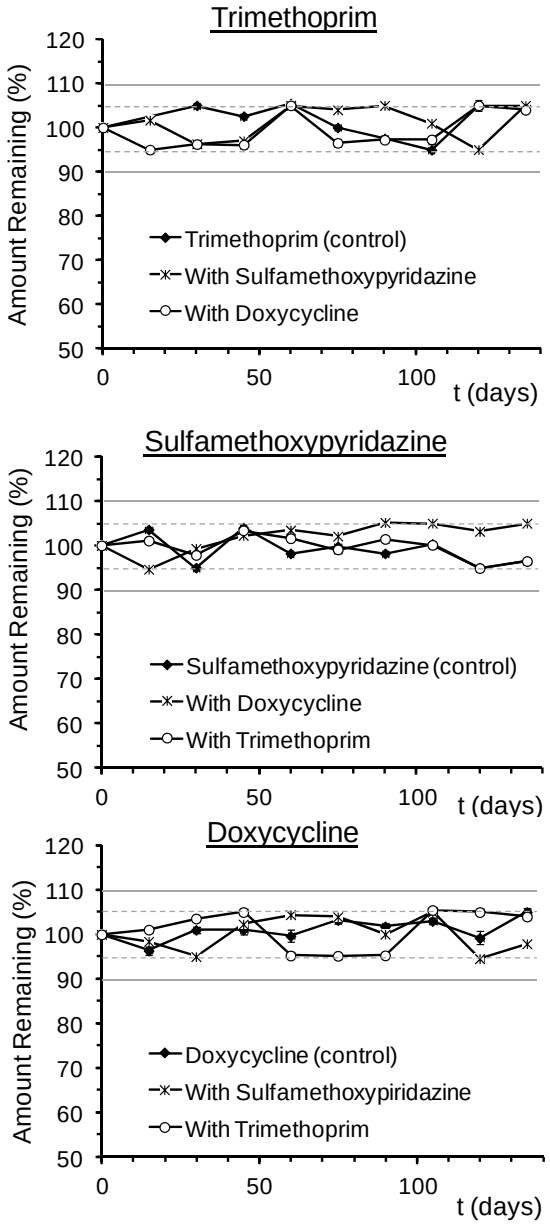

Fig. 1: Stability profile of each drug alone or in combination up to 135 days. The lines indicate the ±5% (dotted line) and the ±10% (solid line) based on ICH 1QA (R2) criteria.

Table 1: Composition of each combination formulation of doxycycline (D), sulfamethoxypyridazine (S) and trimethoprim (T) and the HPLC method used for their simultaneous determination

Code	Composition (%)			HPLC method	Rt (min)
	D	S	T		
D1	100	-	-	II	2.2
S1	-	100	-	II	1.8
T1	-	-	100	I	1.9
S2 D2	23	77	-	II	1.8, 2.2
T3 S2	-	83	17	I / II	1.9 /1.8
T3 D3	60	-	40	I / II	1.9 / 2.2

2013; Paphitou, 2013). These results may encourage the development and application of drug combinations for infection control in the avian, porcine, cattle livestock industries (Langston, 2003). Furthermore, equestrian sports and equine breeding industries may also benefit from combined trimethoprim with sulfadiazine followed by

Table 2: Validation parameters of the HPLC assay for the simultaneous determination of doxycycline, sulfamethoxypyridazine and trimethoprim (CV: coefficient of variation; RE: relative error)

Drug	Nominal QC Conc. (µg/ml)	Intraday assay			Interday assay		
		Experimental concentration (µg/ml)	CV (%)	ER (%)	Experimental concentration (µg/ml)	CV (%)	ER (%)
Doxycycline	30	30.0±0.4	1.2	-0.1	30.2±0.7	2.4	0.6
	60	60.1±0.7	1.2	0.2	59.0±1.4	2.4	-1.8
	90	91.5±1.0	1.0	1.7	90.2±0.7	0.7	0.2
Sulfamethoxy-pyridazine	100	99.2±0.8	0.9	-0.8	100.8±1.9	1.9	0.8
	200	201.9±5.1	2.5	1.0	199.3±3.0	1.5	-0.4
	300	299.0±4.7	1.6	-0.4	300.5±1.5	0.5	0.2
Trimethoprim	5	4.98±0.06	1.10	-0.4	4.94±0.08	1.7	-1.2
	15	15.0±0.1	0.68	-0.1	15.1±0.1	0.7	0.7
	30	30.0±0.3	1.08	-0.1	30.0±0.1	0.3	-0.3

doxycycline in a 12-week treatment protocol against *Burkholderia (B.) mallei*, glanders disease (Saqib *et al*., 2012; Hughes *et al*., 2013). Similarly, treatment of opportunistic infections (e.g. *canine pyoderma*) in pets may also benefit of these combined formulations or enhance its efficacy (Messinger and Beale, 1993).

Conclusion: This study showed the solid-state stability of three antimicrobial drug combinations widely use in veterinary medicine. Trimethoprim, doxycycline and sulfamethoxypyridazine alone or their binary combinations proved stable up to 135 days under controlled storage conditions. These findings may open additional treatment options prepared extemporaneously to improve infection control and safety along the food chain and those involve in production of farmed animals.

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