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Differences between USP and BP dissolution results for oxytetracycline capsules after accelerated stability testing

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The aim of this investigation was to determine whether there was any difference in the dissolution profiles of generic oxytetracycline hydrochloride capsules after storage at accelerated conditions as required in the ICH guidelines on stability testing [1]. In particular, dissolution results measured with the methods described in the USP and BP were analyzed and compared. In South Africa, oxytetracycline is identified as an essential drug with many generic equivalents available. Stability testing is performed on these products to assure their quality [2]. According to the ICH guidelines on stability testing products must be exposed to accelerated testing at 40 °C + 75% relative humidity for three months [1]. During this time dissolution testing is performed to help control the quality of drug products. There is a dissolution test for oxytetracycline in both the USP [3] and the BP [4]. These tests help to assure the consistency of the preparations with respect to its drug release properties because dissolution performance may be an indicator of potential bioavailability or bioequivalency problems [5, 6]. Various investigators have reported that commercial oxytetracycline hydrochloride capsules produced by different manufacturers, are not biologically equivalent [7, 8]. Brice and Hammer [7] performed disintegration and dissolution tests and found that, in general, batches that gave poor serum levels also had slower in vitro dissolution rates. Further investigations [8, 9] on several samples of commercial 250 mg oxytetracycline dihydrate tablets showed that the dissolution rate varied between generic brands obtained from different manufacturers and also between and within batches from one source.

Table: Initial dissolution results compared to dissolution results obtained after 3 months at 40 °C + 75% RH

Product	USP (% dissolved with 60 min)		BP (% dissolved within 45 min)	
	Initial	3 months	Initial	3 months
A	99 ± 5.4	88 ± 1.2	100 ± 1.0	104 ± 1.4
B	96 ± 1.7	96 ± 2.3	105 ± 3.7	95 ± 4.2
C	93 ± 0.1	67 ± 8.5*	106 ± 6.0	90 ± 4.3
D	100 ± 1.8	54 ± 3.0*	102 ± 2.8	102 ± 9.8
E	100 ± 0.8	93 ± 3.0	105 ± 0.5	101 ± 3.1
F	100 ± 0.6	99 ± 0.8	104 ± 1.4	102 ± 0.6

Tolerance for USP is 80% dissolved within 60 minutes and BP 70% dissolved within 45 min
* Products did not comply with USP tolerance

For this study six generic oxytetracycline hydrochloride capsules available on the South African market were purchased from a local pharmacy and subjected to accelerated stability conditions (40 °C + 75% RH) for a period of three months. The capsules were numbered from A–F and dissolution results are the mean of three dissolution tests. Dissolution profiles were compared using a mathematical method described by Moore and Flanner [10]. The equation used to calculate the similarity factor, f_2, is

$$f_2 = 50 \cdot \log\left(\left[1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n} w_t (R_t - T_t)^2\right]^{-0.5} \cdot 100\right)$$

where n is the number of dissolution time points, R_t and T_t are the reference and test dissolution values at time t, respectively, and w_t is an optional weighting factor. The value of f_2 is 100 when the test and reference mean profiles are identical. Values greater than 50 indicate that dissolution profiles are similar. There is no similarity between profiles for values less than 50. For the dissolution of oxytetracycline hydrochloride capsules the USP 24 [3] prescribes water as the dissolution medium using the paddle method at a stirring speed of 75 rpm and the release requirement is that not less than 80% of the labeled amount of oxytetracycline hydrochloride should be dissolved in 60 min. The concentration in solution is calculated from the UV absorbance at 273 nm. The BP 2000 [4] prescribes a tolerance of not less than 70% of oxytetracycline hydrochloride dissolved in 45 min in 0.1 M HCl using the basket method at 100 rpm. The percentage in solution is determined from the UV absorbance at 353 nm. After 3 months stability testing at 40 °C + 75% RH two changes were observed in the appearance of the oxytetracycline capsules. First, a change in the color of the capsule content from yellow to brownish-yellow was seen and secondly the shells of all capsules, except product B, became sticky. However, the mean initial assay for all capsules was 104.31 ± 3.20% and after three months 102.46 ± 2.82%, all within the USP and BP specifications. The dissolution results, summarized in the Table, for all the capsules complied with either the USP and BP dissolution specifications at time 0. At time 0 the mean dissolution profiles of capsules B and C obtained with the USP method were significantly different from that obtained using the BP method ($f_2 < 50$). After 3 months at 40 °C + 75% RH two products, C and D, failed the USP dissolution test because less than 80% of the drug dissolved within 60 min (Fig.). In contrast after 3 months, the

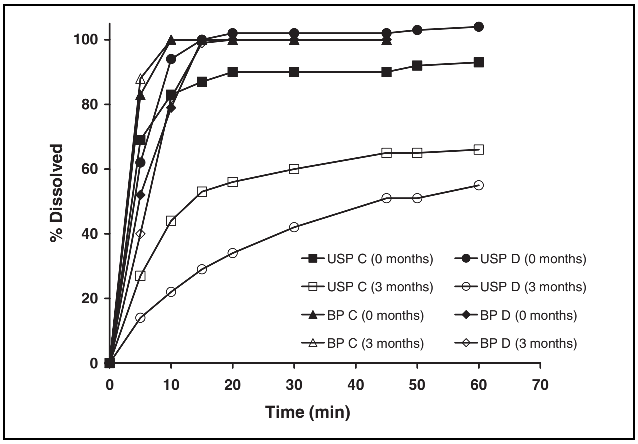

Fig.: Dissolution profiles for oxytetracycline products C and D showing the inability of the BP test to detect the decrease in dissolution after 3 months storage at 40 °C + 75% RH dissolution results for all the capsules complied with the BP specification requiring 70% of the drug to be dissolved within 45 min. Using the test described by Moore and Flanner [10] there was also no similarity found between the mean dissolution profiles of the capsules measured with the USP and BP methods after 3 months' accelerated storage. For the USP test, the mean dissolution profile after 3 months' accelerated storage of only capsule B was comparable to that at time 0. However, for the BP test the mean dissolutions profiles for all the products after 3 months' accelerated storage were not significantly different from that at time 0.

These results show that accelerated stability testing leads to physicochemical changes in oxytetracycline capsules and that the BP dissolution method for oxytetracycline hydrochloride capsules was not able to measure the effect of these changes on the dissolution properties of the capsules. Based on previous reports [7, 8] describing bioavailability problems with oxytetracycline capsules, relying on the BP dissolution test might lead to the acceptance of clinically unacceptable and bio-inequivalent products.

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A new triterpene glycoside from *Zygophyllum eichwaldii*

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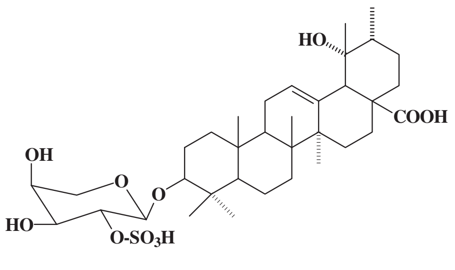
In our continuing studies on the triterpene glycosides from *Zygophyllum eichwaldii* C.A.M. (Zygophyllaceae) [1, 2] we have isolated the new glycoside zygoeichwaloside G (**1**) from the methanol extract of the roots of the plant. Here we report about isolation, structure elucidation and biological tests of the extract and of isolated compound.

Chromatography of the methanol extract on silicagel column afforded the glycoside **1** as an amorphous substance. Acidic hydrolysis of the glycoside yielded pomolic acid as aglycone and arabinopyranose as sugar component. The ^{13}C NMR spectrum (Table) contained 35 different signals suggesting that the glycoside **1** is a monoside. This is confirmed by availability of hydrogen anomeric atom at δ 5.16 and carbon atom at δ 103.59 in the ^{1}H and ^{13}C NMR spectra. The signal of carbon atom of the (COOH) group at δ 180.68 showed that it is free, and that arabinose is linked to one of the aglycone hydroxyls. The ^{13}C NMR spectrum of zygoeichwaloside G (**1**) contained signals at δ 89.41 attributable to C-3 and showing that the hydroxyl group at this carbon is glycosylated. Consequently, arabinose is located at C-3 of pomolic acid. The conclusion was confirmed by HMBC and ROESY spectra, in which the correlation was observed among anomeric protone (H-1) of arabinose and C-3 and H-3 of aglycone. The value of CSSI (5 Hz) corresponds to α-configuration of glycosidic bond.



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The NMR data of zygoeichwaloside G are very similar to those of zygoeichwaloside C (**2**) isolated earlier [1]. Comparison of the ^{1}H and ^{13}C NMR spectra of the compounds **1** and **2** showed that the aglycone parts of these compounds concided. But there is a considerable difference between the chemical shifts of protons and carbon atom signals which belong to arabinose (Table). By COSY, TOCSY and HSQC experiments it was established that arabinose at C-2 is substituted and that the substituent does not contain carbon atoms. The substituent was electronegative enough to cause characteristic shifts