In vitro dissolution of expired antibiotics

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The most common pharmaceutical formulations for antibiotics are the tablets and hard gelatin capsules. The typical shelf-life of these dosage forms is five years. In the most countries of the world it is required by the law that the expired medications have to be disposed following the respective regulations. Furthermore, it is common sense not to use the expired medications. The present study was intended as an academic investigation only and it was aimed to examine the *in vitro* dissolution performance of at least 10 years expired amoxicillin, ampicillin, or doxycycline tablets or hard gelatin capsules. The dissolution tests were performed using the Apparatus 2 technique as described in the US Pharmacopoeia (USP 35/ NF30) respective monographs. For the comparison, the non-expired antibiotics registered in European Union (EU) and available in the community pharmacies of Estonia were used. The respective USP tolerance limits for the dissolution tests were applied to qualify the formulations tested as "passed" or "failed". Expectedly, all EU registered non-expired formulations passed the USP test. But all tablets and hard gelatin capsules of the expired antibiotics passed the USP tests as well. In conclusion, our results demonstrate that the shelf-life of antibiotics in the dosage forms of tablets or hard gelatin capsules is underestimated what concerns their *in vitro* dissolution performance. However, the authors in no way promote the use of expired medications, neither was it completely out of the study to give any hint on the therapeutic value of these expired medications.

Keywords amoxicillin; ampicillin; doxycycline; expiration; *in vitro* dissolution

1. Introduction

The expiration of medical formulations is a sensitive and much debated topic. The GMP (good manufacturing practice) rules of pharmaceutical industry as well as the national (and international) regulatory agencies such as the European Agency of Medicines or Food and the Drug Administration strictly control the quality of registered medications throughout the shelf-life. The shelf-life of medicines depends on the dosage form used and it is a common approach that for the tablets and hard gelatin capsules it has to be five years [1]. In the most countries of the world the applicable regulations foresee that the expired formulations have to be disposed in line with the local legislation. Indeed, it is common sense not to use expired medications. However, in certain circumstances such as the humane catastrophes, wars, financial collapse, high risk of pandemics, *etc*. the question remains whether the medications available but expired have retained the sufficient pharmaceutical quality to be used in case where simply no alternatives exist? Furthermore, it is evident that most of the active pharmaceutical ingredients (API) themselves retain their pharmacological potency far beyond the expiration date and the focus of the medications' expiration is rather in the preservation of the *in vivo* performance of the respective formulations. The situation with the expired medications is well illustrated with an example from the 2010 regarding the risk of the outbreak of the pandemic of the avian flu. The FDA stated that "Eighteen lots of Tamiflu Capsules and three lots of Relenza Inhalation Powder have been authorized by FDA for use beyond their expiration dates. FDA has authorized the use of these lots beyond their expiration dates under Emergency Use Authorizations (Tamiflu EUA and Relenza EUA) that specify certain conditions of use. FDA's decision to authorize the use of these lots beyond their expiration dates is supported by FDA's approval of applications submitted by the manufacturers of Tamiflu and Relenza that extended the expiration dates of certain Tamiflu and Relenza products [2]."

The most common pharmaceutical formulations for antibiotics are the tablets and hard gelatin capsules while the conventional pattern of the API release from the formulations is preferred. The dissolution test is a golden standard to measure the *in vitro* performance of the tablets and hard gelatin capsules and nowadays the dissolution test techniques described in the US Pharmacopoeia (USP) are in certain cases accepted as the substitutes for the *in vivo* bioavailability studies [3]. Numerous post-marketing studies have utilized the *in vitro* dissolution test to investigate the amount of API released und thus, have evaluated the various aspects of the pharmaceutical quality of the oral solid dosage forms of antibiotics [*e.g*., for a review on substandard and counterfeit antibiotics, see: 4]. However, regarding the expired medications the research has focused rather on the API degradation and therefore, the knowledge of the formulation performance is limited. The pharmaceutical quality of the expired medications is a especially hot topic in emerging countries, however, the data from those countries are incomplete. As a peculiar case, Prazuck *et al*. [5] attempted to characterize the expired chlortetracycline from Northern Myanmar, but it appeared that the formulations did not contain the chlortetracycline at all. Furthermore, the unidentified API elicited no antimicrobial activity, either [5].

The present study was intended as an academic investigation only and it was aimed to examine the *in vitro* dissolution performance of at least 10 years expired amoxicillin, ampicillin, or doxycycline tablets or hard gelatin capsules. For the comparison, the non-expired antibiotics registered in European Union (EU) and available in the community pharmacies of Estonia were used. In our former studies on solid dosage forms of antibiotics we have found

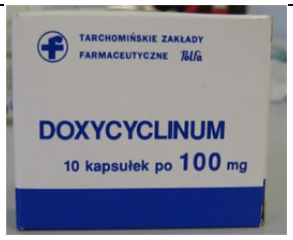
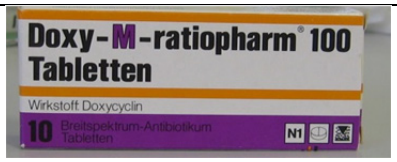
that the non-expired, EU registered antibiotics always were in the dissolution test tolerance limits given of the respective USP monographs [6,7]. Since no information was available how the antibiotics studied were tested years ago, the head-to-head comparison with the non-expired medications indirectly verified that the dissolution test parameters chosen were appropriate for the formulations selected.

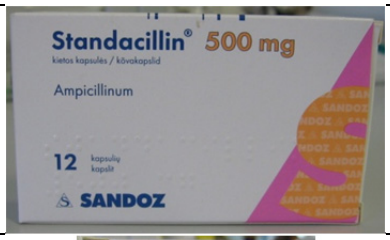
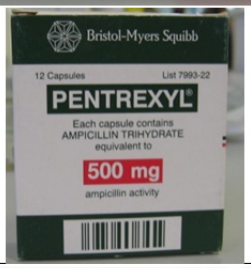
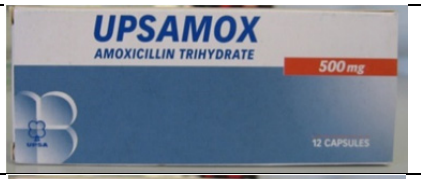
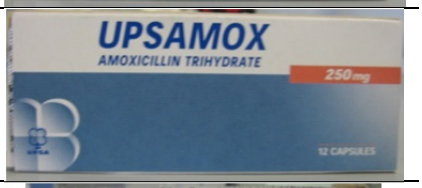
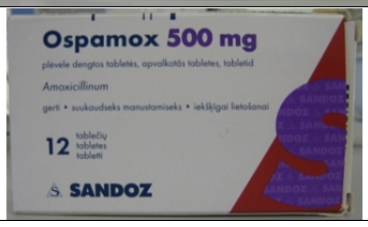
2. Materials and methods

2.1. Formulations

Twelve different conventional API release formulations were tested: four doxycycline, three ampicillin, and five amoxicillin formulations. The EU-registered and non-expired reference formulations were purchased from the University of Tartu Pharmacy, Tartu, Estonia. The expired formulations were obtained numerous years ago via distinct suppliers and these formulations were stored at the Department of Pharmacy, University of Tartu in a closed cupboard at ambient room temperature (20±2°C) and relative humidity. The exact details of the purchase of those formulations could not be verified afterwards. Some of the packages of the plastic vials of the expired formulations were opened and closed again, but none of the tablets or capsules tested was during the storage years directly exposed to the environment. The formulations in blister packages were not opened. No distinctions were made in data interpretations regardless the packages were opened beforehand or not. The hard gelatin capsules of Upsamox were in forms of two distinct strengths (250 and 500 mg). The list of formulations tested is given in the Table 1.

Table 1 List of formulations tested.

Brand/Formulation	Manufacturer	Lot Nr.	Expiration date	Package image
Apo-Doxy	APOTEX INC. Toronto, Canada	W5020	05.1995	
Doxycyclinum	Trachominnskie zakkady farmaceutyczne, Poland	120195	01.02.1999	
Doxy-M-ratiopharm ® 100 Tabletten	Ratiopharm GmbH	0269B2	31.12.1994	
Doxy-M-ratiopharm ® 100 mg	Ratiopharm GmbH	K52618	11.2015	
Apo-Ampi	APOTEX INC. Toronto, Canada	KB0333	12.2000	

Standacillin ® 500 mg	Sandoz GmbH	AR7621	11.2013	
Pentrexyl ®	Bristol-Myers Squibb	MM6375	12.1999	
Moxilen 250	Medochemie Ltd.	L8354	06.1997	
Amoxicillin-ratiopharm 500 mg	Ratiopharm GmbH	J43457	11.2013	
Upsamox	Bristol-Myers Squibb	9B15083	12.2001	
Upsamox	Bristol-Myers Squibb	8I10882	07.2001	
Ospamox 500 mg	Sandoz GmbH	150049	08.2011	

2.2. Dissolution test

All non-expired formulations were tested several months before the expiration date, except the Ospamox tablets (expiration 08.2011) that were tested in July, 2011. The dissolution test methods described in the US Pharmacopoeia (USP35/NF30) respective monographs were used with some modifications. The Apparatus 2 technique was employed, but always the paddle method was used for tablets and the basket method for hard gelatin capsules. The respective USP monographs tolerance limits were employed. Thus, the particular formulation either “passed” or “failed” the test.

All dissolution tests were carried out in 900 ml of purified water medium at 37°C using the SOTAX AT 7 dissolution test equipment connected to Ultrospec III (Pharmacia LKB) spectrophotometer and fed by the Watson-Marlow 202 U/AA peristaltic pump. The system was driven by a custom adapted Win TDS computer program.

The speed of rotations and wavelengths at which the measurements were taken were variable: 75 rpm and 276 nm for doxycycline, 100 rpm (250 mg hard gelatin capsules) or 75 rpm (500 mg hard gelatin capsules) and 272 nm for amoxicillin, and 100 rpm and 254 nm for ampicillin.

From each formulation, 12-18 tablets or hard gelatin capsules were tested. The dissolution tests were carried out in the second half of 2011.

3. Results and discussion

Expectedly, all non-expired, EU registered formulations passed the USP test (Table 2). Estonia belongs since 2004 to the EU and far before the European drug quality criteria were implemented in Estonia. Though formally the USP is not a legislative instrument for Estonia, Estonia as an EU member belongs to International Conference on Harmonization and thus the USP tests and assays *de facto* satisfy the Estonian regulatory norms. As noted above, in our former studies we tested the *in vitro* dissolution performance of the antibiotics' formulations from the Russian Federation and those available in Estonian drug market, and always the Estonian drug market antibiotics performed excellently [6,7]. This result was predictable: though the substandard medications are of concern everywhere, in the industrialized countries their prevalence has been estimated by the WHO to be very low [8].

Table 2 In vitro dissolution performance of formulations tested.

Formulation	Dosage form	USP tolerance limit	USP tolerance limit passed	USP norm for API content
Apo-Doxy	hard gelatin capsule	80% 30 min*	yes	90-120%
Doxy-M-ratiopharm	tablet	85% 90 min	yes	90-120%
Doxycyclinum	hard gelatin capsule	80% 30 min*	yes	90-120%
Doxy-M-ratiopharm	tablet	85% 90 min	yes	90-120%
Upsamox 500 mg	hard gelatin capsule	80% 60 min	yes	90-120%
Upsamox 250 mg	hard gelatin capsule	80% 60 min	yes	90-120%
Moxilen 250 mg	hard gelatin capsule	80% 60 min	yes	90-120%
Amoxicillin-ratiopharm 500 mg	(polymer-coated) tablet	70% 90 min	yes	90-120%
Ospamox 500 mg	(polymer-coated) tablet	80% 60 min	yes	90-120%
Standacillin	hard gelatin capsule	75% 45 min	yes	90-120%
Pentrexyl	hard gelatin capsule	75% 45 min	yes	90-120%
Apo-Ampi	hard gelatin capsule	75% 45 min	yes	90-120%

* USP Doxycycline Hyclate Capsules monograph

The major result of our study is that the formulations of antibiotics expired long time ago showed *in vitro* dissolution performance comparable to those available on drug market today (Table 2, Fig 1). In an extensive review of Lyon *et al*., [9] where data from the shelf-life extension program (SLEP) were assessed, the 122 drug products evaluated were categorized into five groups based on shelf-life extension data (according to the relative number of lots initially extended and number of extended lots terminated). In line with this review the doxycycline, amoxicillin, and ampicillin belong to the groups 1A and 1B, meaning their expiration dates could be extended. Our study confirms these results. Indeed, putting the conclusion of research by Lyon *et al*., [9] into the context of former research data where the quality of medications beyond their expiration dates was assessed [10-12], our study demonstrates that at least some of the antibiotics might be, if properly stored, extended past the expiration date what concerns their *in vitro* dissolution performance. However, the authors in no way promote the use of expired medications, neither was it completely out of the study to give any hint on the therapeutic value of these expired medications. Further studies are essential to verify the clinical efficacy of the expired antibiotics.

3. Conclusion

In conclusion, the present independent, academic only study further provides evidence that the expiration dates of solid dosage forms of classic antibiotics are sometimes considered far too conservative. In view of the possible cost-savings the reconsideration of the shelf-life of some antibiotics might have general beneficial effect, especially for the source limited countries. However, further case-by-case research is essential. At the present, no sufficient efficacy or any other clinical data are available for the expired medications, so one could not extend the shelf-lives of antibiotics purely on an administrative basis without the appropriate experimental proofs.

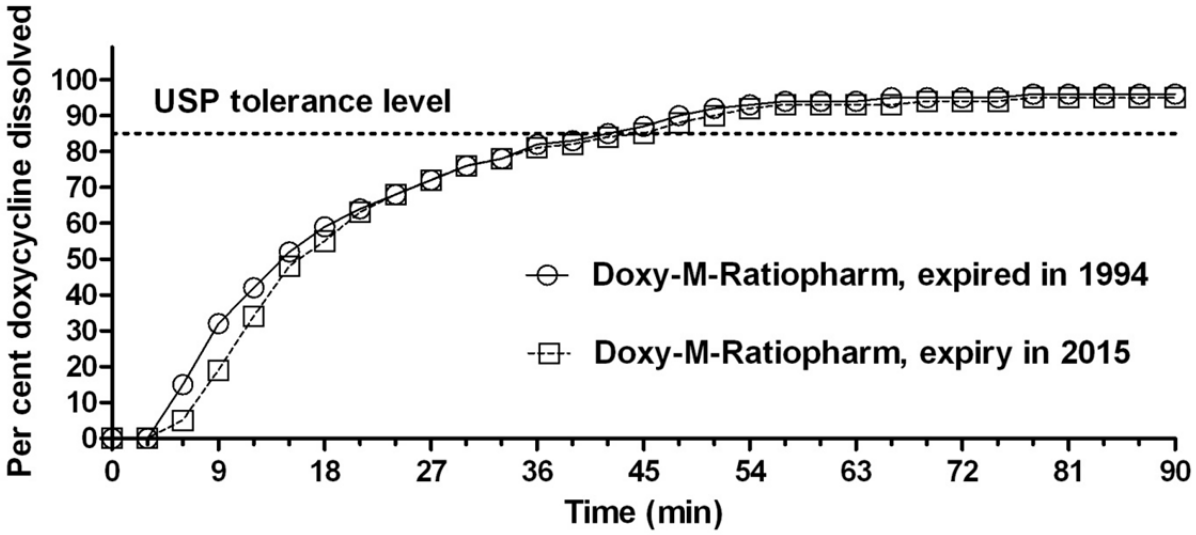


Fig. 1 Almost identical *in vitro* dissolution curves of 17 years expired and non-expired doxycycline tablets from the same manufacturer (Ratiopharm GmbH), the dissolution tests were carried out in late 2011. Each symbol represents an average of 12 tablets tested.

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