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Hydrolysis of sulphonamides in aqueous solutions

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ABSTRACT

Hydrolysis is one of the most common reactions controlling abiotic degradation and is one of the main paths by which substances are degraded in the environment. Nevertheless, the available information on this process for many compounds, including sulphonamides (a group of antibiotic drugs widely used in veterinary medicine), is very limited. This is the first study investigating the hydrolytic stabilities of 12 sulphonamides, which were determined according to OECD guideline 111 (1st category reliability data on the basis of regulatory demands on data quality for the environmental risk assessment of pharmaceuticals). Hydrolysis behaviour was examined at pH values normally found in the environment. This was prefaced by a discussion of the acid–base properties of sulphonamides. All the sulphonamides tested were hydrolytically stable at pH 9.0, nine (apart from sulphadiazine, sulphachloropyridazine and sulphamethoxypyridazine) were stable in this respect at pH 7.0 and two (sulphadiazine and sulphaguanidine) at pH 4.0 (hydrolysis rate ≤ 10%; $t_{0.5\,(25\,^{\circ}C)}$ > 1 year). The degradation products were identified, indicating two independent mechanisms of this process. Our results show that under typical environmental conditions (pH and temperature) sulphonamides are hydrolytically stable with a long half-life; they thus contribute to the on-going assessment of their environmental fate.

1. Introduction

The majority of the earth's surface is covered by water in the form of oceans, seas, lakes or rivers. Hence, chemical pollutants (e.g. pharmaceuticals) entering the environment are usually degraded via hydrolysis [1]. Because water is present in great excess compared to the concentrations of the chemicals, this type of reaction is usually described as a pseudo-first order reaction at fixed pH and temperature, and may be influenced by acidic or basic species H_3O^+ and OH^-. It has been established, that many of the most frequently applied penicillins are difficult to detect in the environment because they are hydrolysed, especially in alkaline sewage [2,3].

Determination of the hydrolysis behaviour of pharmaceuticals is especially important in the case of poorly or non-biodegradable substances. This is because, for example, the presence of antibiotics in the environment may elicit the development of antibiotic-resistant genes in microorganisms, which can be transferred to human beings and animals through food chains and drinking water, resulting in the failure of antibiotic treatment of infections [4,5]. On the other hand, when pharmaceuticals degrade in the environment, they may form persistent and toxic transformation products, which should be accounted for in the environmental risk assessment (ERA) of the parent compounds [6]. Even though hydrolysis is known to be one of the most common chemical reactions controlling stability and is, therefore, one of the principal chemical transformation pathways of these substances in the environment, literature data on the hydrolytic stabilities of pharmaceuticals are very limited [7].

In order to arrive at reliable ERAs, suitable data on the environmental exposures and ecotoxic potencies of compounds are needed [8]. Regulatory demands on data quality for the ERA of veterinary pharmaceuticals are given in the guideline on environmental impact assessment for veterinary medicinal products [9]. According to the European Medicines Agency (EMEA) and the Food and Drug Administration (FDA), the laboratory test method for assessing abiotic hydrolytic transformations of chemicals in aquatic systems at pH values normally found in the environment (pH 4–9) should be based on OECD Guideline 111 [10,11].

Sulphonamides (SAs) are antibiotics that have been widely used in veterinary medicine for almost fifty years [12,13]. They are incompletely metabolised and are excreted partly as unchanged parent compounds and partly as metabolites [14]. They enter the ecosystem from wastewater discharge, manure disposal, aquaculture and animal grazing [15], and may be later transported to various environmental compartments such as surface water,

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ground water, soil or sludge. Sukul and Spiteller [16] noted that during the storage of manure, the excreted acetyl conjugates could be cleaved back to the parent compounds. SAs are only partially removed by conventional WWTPs [17,18], and are found in surface water (in the ng L^{-1} to μg L^{-1} range) and ground water (ng L^{-1}) [e.g. 19,20]. Such distribution may have significant and long-term effects on the rate and stability of ecosystem functioning [12,21–24].

The literature data concerning the hydrolytic stabilities of sulphonamides are contradictory. On the one hand, they indicate that these antibiotics are resistant to hydrolysis [25–28], on the other, they claim that they are degradable, especially in acidic solution [29–32]. Moreover, the hydrolysis rates were calculated using procedures other than OECD 111.

In this work, the hydrolysis behaviour of 12 sulphonamides was investigated according to OECD 111. The formation of selected degradation products – aniline, sulphanilic acid and sulphanilamide – was monitored and the mechanism of sulphonamide hydrolysis discussed. Reliable hydrolytic stability data for all the tested SAs were obtained.

2. Materials and methods

2.1. Chemicals

All sulphonamides (Table 1), apart from sulphadimidine and sulphapyridine (Serva, Heidelberg, Germany), were purchased from Sigma–Aldrich (Steinheim, Germany). The compounds used for preparing buffer solutions (all analytical grade) – monopotassium phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), potassium chloride (KCl) and boric acid (H_3BO_3) – were obtained from POCH (Gliwice, Poland), and citric acid ($C_6H_3O_7 \times H_2O$) and sodium hydroxide (NaOH) were from Stanlab (Lublin, Poland). Deionised water was produced by the HYDROLAB System (Gdansk, Poland). The acetonitrile (ACN) (HPLC grade) used for the mobile phases and for preparing the comparative standard solutions was obtained from POCH S.A. (Gliwice, Poland). Trifluoroacetic acid 99% (TFA) (for mobile phase acidification) was purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. The hydrolytic stabilities of 12 sulphonamides determined according to OECD 111

The hydrolytic stabilities of the SAs were determined according to the method set out in OECD 111 [10]; the scheme is shown in Fig. 1. The studies were conducted in capped glass vials, under dark and sterile conditions. Before and after degradation, the samples were analysed using a validated HPLC-UV method [33] in order to quantify the test substances and hydrolysis products. All tests were done in two replicates. Chromatographic separations were performed using a high performance liquid chromatograph (Perkin Elmer Series 200) consisting of a chromatographic interface (Link 600), binary pump, UV/vis detector, vacuum degasser and Rheodyne injection valve. SA samples were separated on a Gemini C18-110A column (150 mm × 4.6 mm, 5 μm, Phenomenex Inc. Torrance, CA) (room temperature, wavelength 270 nm, injection volume 50 μL, flow rate 0.7 mL min^{-1}). Mobile phase A was H_2O with the addition of TFA at pH 3.5, and mobile phase B was 100% acetonitrile; both were selected in the gradient programme. Elution began with 90% of mobile phase A, which was reduced to 40% within 20 min. Chromatographic separation of SAs and degradation products was achieved within less than 20 min.

2.2.1. Test conditions

Experiments were performed at pH 4.0, 7.0 or 9.0.

Buffer solution pH 4.0 was prepared from aqueous solutions 0.2 M K_2HPO_4 and 0.1 M citric acid mixed in the ratio 38.55/61.45 (v/v). The pH 7.0 buffer consisted of 0.1 M NaOH, 0.1 M KH_2PO_4 and water in the ratios 29.63/50.00/20.37 (v/v/v), and the pH 9.0 buffer of 0.1 M NaOH, 0.1 M H_3BO_3 in 0.1 M KCl and H_2O in the ratios 21.30/50.00/28.70 (v/v/v). The pH of each buffer solution was checked with a CP-411 laboratory pH-metre (Elmetron-Zabrze, Poland) to an accuracy of at least 0.1 at the required temperature. Next, the buffer solutions were passed through 0.2 μm fibreglass (Chromafil® 148 PET 15/25, Marchery-Nagel, Düren, Germany), bubbled by nitrogen for 5 min (to avoid oxygen) and thermostated at the required temperature before the experiment.

2.2.2. Performance of the test

2.2.2.1. Preliminary test (Tier 1). On the day of the experiment, a stock solution of the sulphonamide to be analysed (100 mg L^{-1}) was added to the appropriate buffer solution (bubbled again by nitrogen for 5 min) to a concentration of SA 1 mg L^{-1}. The solution obtained was divided into two portions: the first portion was subjected to HPLC-UV analysis, whereas the second one was transferred to a 10 mL glass vial (typical of the headspace technique) and stored in a capped vial under dark and sterile conditions at the required temperature and for the requisite length of time (Fig. 1) (Incubator ICT 5.4, Falc, Treviglio, Italy). Additionally, on the day of the experiment, the control solution (1 mg L^{-1}) of SA in a mixture of H_2O:ACN (90:10, v/v) was prepared. It was stored at 4 °C, then analysed by HPLC-UV on the first day of the experiment; subsequently, the degraded sample was likewise subjected to HPLC-UV analysis on selected days (Fig. 1). This procedure was applied to each sulphonamide.

Determination of the hydrolysis rate of the SAs was based on Eqs. (1)–(3):

$$S^0 = \frac{P^0}{M^0} \times 100 \quad (1)$$

$$S^t = \frac{P^t}{M^t} \times 100 \quad (2)$$

$$S^0 = S^0 - S^t \quad (3)$$

where S is the SA hydrolysis rate [%], S^0 is the hydrolysis rate of SA before hydrolysis [%], S^t is the hydrolysis rate of SA determined for a time t degraded sample [%], P^0 is the chromatographic peak area of SA determined for a non-degraded sample on the day of the experiment, P^t is the chromatographic peak area of SA after sample degradation at fixed pH, temperature and time, M^0 is the chromatographic peak area of SA determined for the control solution on the first day of the experiment, M^t is the chromatographic peak area of SA determined for the control solution kept for time t at 4 °C.

2.2.2.2. Hydrolysis of unstable substances (Tier 2). The higher tier test was performed at the pH values at which the test substance was found unstable, as defined by the preliminary test above (Fig. 1). The buffered solutions of the test substance were thermostated at selected (20 °C, 40 °C and 70 °C) temperatures. To test the hydrolysis rate as a function of pH and temperature, each reaction was allowed to proceed for 30 days, and individual replicate test samples (in separate reaction vessels) were analysed by HPLC-UV ($n = 3$) at each of six sampling times. Next, for each hydrolytically unstable SA, the following function (4) was applied in order to test kinetic behaviour:

$$k_{obs} = \frac{1}{t} \times \ln \frac{C_t}{C_0} \quad (4)$$

where k_{obs} is the pseudo first-order hydrolysis rate constant at fixed temperature ($time^{-1}$), C_0 and C_t are the respective concentrations of the SA at time zero and t, ln is the Naperian logarithm. For each sulphonamide, the logarithms of the concentrations $\ln C_t/C_0$ were plotted against time (t) and the slope of the resulting straight line