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Stability of adenosine in infusion

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Abstract

Using liquid chromatography the stability of adenosine in aqueous solution was investigated at five different temperatures, namely 4, 22, 37, 60 and 72°C over a period of 6 months. At the three lowest temperatures, the initial concentration of the product was not altered, at the highest temperatures there was a significant decrease. From these data the shelf life (t_{90}) at room temperature was estimated to be at least 5 years. © 1998 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Adenosine (Ado) is an endogenous nucleoside which is a degradation product of adenosine triphosphate (ATP). The general pharmacological effects were described in the late 1920s by Drury et al. [1]. Ado interacts with the adenosine receptors A_1 and A_2 on the surface of different cell types. The effects of Ado have been reviewed by many authors [2]. In humans, Ado is metabolized in almost all cell types, including the endothelial cells of blood vessels. It is phosphorylated into adenosine monophosphate (AMP), after which it is deaminated to inosine and then to xanthine [3]. Finally, different metabolic pathways lead to uric acid which is excreted into the urine.

Ado has been used for treatment of paroxysmal supraventricular tachycardia [4], and non-invasive assessment of coronary artery disease [5]. Furthermore, it can be used for the diagnosis of different types of tachycardia [6].

Ado is administered under the form of an injectable solution, mostly in a hospital environment. Up until very recently no commercial preparation was available and many hospitals prepare their own Ado solutions. A common problem is the stability of these self-made solutions. The literature on this subject is very scarce and does not provide practical data [7]. The purpose of this study is therefore to determine the stability of Ado in an aqueous physiological salt solution at room temperature. It should indeed be mentioned that crystallization of Ado in the infusion prevents storage at temperatures lower than room temperature.

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The composition and preparation of the solution studied here were identical to the clinically used solution.

Previous studies on the chemical stability of adenosine were performed in acid [8,9] and alkaline [10–12] media. No data are available on degradation kinetics of adenosine in neutral media.

2. Materials and methods

2.1. Preparation of the solution and samples

The solution was made by dissolving 4.000 g of Ado (Acros Organics, Geel, Belgium) in 2000 ml of a 0.9% (m/v) aqueous solution of NaCl (Irripack B. Braun, Melsungen, Germany). Then, this solution was filtered through an 0.22 μm cellulose nitrate membrane filter (Millipore, Bedford, MA, USA) and a pyrogen eliminating filter (Sartorius, Göttingen, Germany). The solution was collected in plasmavac bottles (Baxter, Lessines, Belgium), which were sterile and under vacuum. Small sterile autosampler vials (approx. 1.5 ml) were then filled with the solution, capped and numbered. They were divided in five groups and stored at 4, 22, 37, 60 and 72°C respectively. Every 2 weeks three samples were taken at each temperature condition and stored in a freezer at -80°C. After six months, all samples were analyzed.

2.2. Chemicals

The LC mobile phase was prepared with double distilled water and distilled methanol. Ado and adenine were from Sigma (St. Louis, MO). All other reagents were of pro analysi quality (Merck, Darmstadt, Germany).

2.3. LC apparatus and operating conditions

The system was composed of a Merck-Hitachi L-6200 intelligent pump (Darmstadt, Germany), a Merck-Hitachi autosampler with a 10 μl loop, a Waters detector model 440 (Milford, MA, USA) set at 254 nm and an integrator model 3396A (Hewlett Packard, Avondale, PA, USA). A Waters model 990 photodiode array detector was used to record on-line UV spectra. A 250×4.6 mm ID column was homepacked with Hypersil C18 5 μm (Shandon, Astmoor, Cheshire, UK). The mobile phase consisted of methanol, 0.2 M potassium phosphate buffer pH 6.0 and water in a ratio of 7:5:88, v/v/v. The flow rate was 1.0 ml min^{-1}. The column was immersed in a thermostated waterbath (Julabo, Seelbach, Germany) at 35°C. The repeatability of the method was satisfactory (RSD = 0.8%, $n = 20$, 2 mg ml^{-1} Ado).

3. Results and discussion

No LC systems have been described for the analysis of adenosine in pharmaceutical formulations. Adenosine has been assayed for biological purposes using reversed phase [13–16] and reversed phase ion-pairing systems [17,18]. In the frame of this study a reversed phase method was used, containing methanol as the organic modifier and potassium phosphate pH 6.0 as the buffer. Analytical parameters calculated for this system were: resolution between Ado and adenine = 4.5 and symmetry for Ado = 2.3. A typical chromatogram is shown in Fig. 1.

The assay was shown to be stability-indicating by peak purity assessment on a degraded sample. Diode array spectra at several positions of the Ado peak show that the peak is pure, so that the percentage degradation can be calculated by reduction of the main peak with time.

Since the infusion was not buffered, and since the pH may affect the reaction rate, the pH of the samples was checked before and after degradation and was found to remain unchanged, namely neutral.

100% of the original Ado concentration was still present after 6 months at 4, 22 and 37°C. Therefore it is proven that an Ado infusion can be stored at room temperature for this period of time. Table 1 lists the percentage of Ado remaining at 60 and 72°C as a function of time. The shelf life at 60°C was graphically deduced to be 250 days. In the same way the shelf life at 72°C was found to be 91 days. From these values it was

possible to estimate the shelf life at room temperature (20°C), which was 36 years [19]. Estimation of shelf lives by extrapolation was allowed because the reaction in neutral media, such as this infusion, is most surely first order, based on literature about adenosine degradation in acid [8,9] and alkaline [10–12] media. The degradation taking place in physiological salt solution at forced conditions of temperature (60 and 72°C) showed adenine to be the main degradation product. The mechanism of breakdown or the nature of the catalytic process in neutral media can not be told from these data. In acid media however, it is known that the base part (adenine) is protonated after which the N-glycosidic bond is broken to form adenine and an intermediate glycosyl oxocarbenium ion. The latter reacts with water to form ribose.

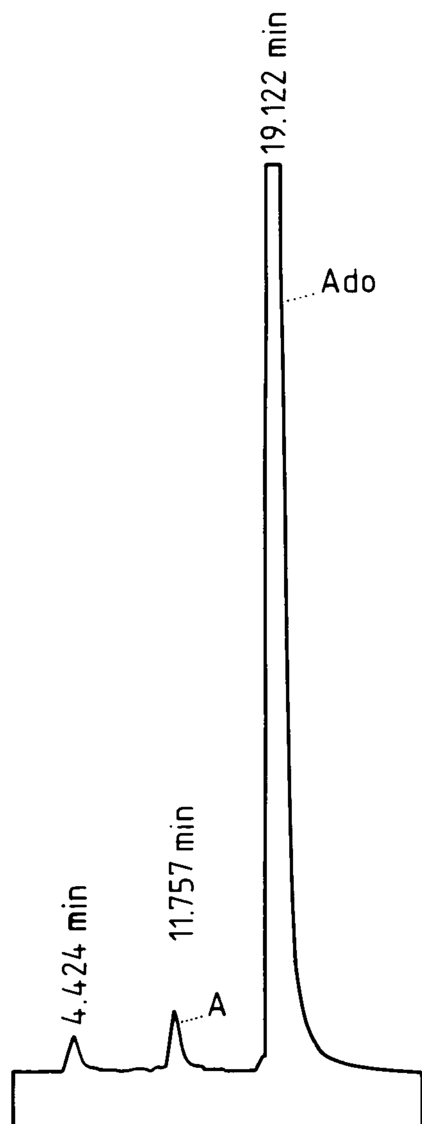

Fig. 1. Typical chromatogram of Ado infusion degraded at 60°C for 56 days. Analytical conditions: Hypersil C18 5 μm, 250 × 4.6 mm I.D.; mobile phase: methanol—0.2 M potassium phosphate buffer pH 6.0—water (7:5:88, v/v/v); flow rate: 1.0 ml min^{-1}; temperature: 35°C; detection: UV at 254 nm; A = adenine.

Table 1
Percentage of Adenosine (Ado) remaining at 60 and 72°C as a function of time

Time (days)	Mean normalized % of Ado	
	60°C	72°C
0	100.0	100.0
14	100.0	97.7
28	99.2	95.4
42	97.8	93.5
56	96.7	91.6
70	96.7	90.7
84	96.1	N/A
98	95.5	87.2
112	94.9	87.6
126	95.2	88.9
140	93.4	85.2
154	94.3	86.6
168	94.1	N/A

N/A: not analyzed.

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