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Development, validation and stability study of pediatric atenolol syrup

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Atenolol [4-(2-hydroxy-isopropylaminopropoxy)-phenylacetamide], is a cardioselective β_1-adrenergic receptor blocking agent prescribed for treatment of hypertension, angina pectoris and cardiac arrhythmias. However, most of these medicines are not formulated for easy or accurate administration to children. Atenolol is unstable in solutions and therefore the development of a liquid dosage form is a significant challenge. Studies showed that the degradation rate of atenolol is dependent on the temperature, indicating higher stability at 4 °C. Atenolol syrup is stable for 9 days, with acceptable apearance. A second order model adequately described atenolol decomposition when stored as syrup. A stability-indicating method was developed and validated in order to evaluate these studies.

1. Introduction

Many drugs frequently used in infants and young children are not available in suitable dosage forms (Nahata 1999). Clinical studies adequate for pediatric labeling of drugs are often more costly, pose patient recruitment challenges, encompass unique ethical and practical issues and involve greater potential liability than comparable studies carried out in adult populations (Freed et al. 2005). Depending on their age, many children are unable to swallow whole tablets or capsules (Standing and Tuleu 2005). As a result, extemporaneous compounding (off label), involves preparing an oral liquid from the pure drug powder (Schirm et al. 2003). However, it is critical to determine the stability of various drugs at clinically important concentration and under practical storage conditions. Presentations and publications on stable drug formulations will offer oportunities for pediatric patients to receive the desired drugs and doses most effectively and safely. Chemical stability is determined immediately after preparation and during storage, using accurate, reproducible, specific, and stability-indicating analytical methods such as high-performance liquid chromatography. The studies are performed simulating clinical conditions for drug concentration, storage temperature and type of container, while using an easily accessible liquid vehicle. At least 90% of the drug should remain active during storage for it to be considered stable (Nahata 1999).

Antihypertensives are an example of a potential need for pediatric extemporaneous solutions. The majority of medicines used for children, acting on the cardiovascular system, are unlicensed (Standing and Tuleu 2005), although β-adrenergic blocking agents are useful in the prevention of several types of arrhythmias in childhood. A β-adrenergic blocking agent with a longer $t_{1/2,}$ such as atenolol, could be advantageous for the pediatric population. Atenolol would possibly allow once- or twice-daily dosing in children because it has a $t_{1/2}$ of 5 to 7 h in adults. Atenolol also has β_1 receptor selectivity, which is another advantage over propanolol for use in asthmatic or hypoglycemic patients (Buck et al. 1989). Despite several publications that adressed atenolol stability (Garner et al. 1994; Nahata 2000), only limited information for syrup vehicles is available.

The objective of present study is to determine the chemical stability of atenolol pediatric syrup, in order to allow pharmacists to assign expiration periods with safety and efficacy.

2. Investigations, results and discussion

2.1. Method validation

The selectivity of the method was determined by comparing the chromatograms obtained from samples containing atenolol with those obtained from simple syrup samples.

The limit of quantification (LOQ) of the developed method was determined by injecting progressively lower concentrations of the standard solution under the chromatographic conditions used. The lowest concentration assayed where the signal/noise ratio was at least 10 : 1 was regarded as the LOQ.

Under the HPLC developed conditions, the limit of quantification determined was 3 ng/mL for three successive injections of the sample.

Standard solutions (8, 9, 10, 11 and 12 μg/mL), each in three replicates, were injected into the system. The curves were constructed using analytical grade atenolol. The linear regression method was used for data evaluation. Linearity was expressed as a correlation coefficient; the value must be >0.999.

The standard curves were linear over the investigated range of 8–12 μg/mL. The correlation coefficient of 0.9989 suggests that the HPLC method developed had good linearity.

The precision of the method was tested by injecting five standard solutions of 20 μg/mL of atenolol (repeatability assay). This assay was repeated for 5 days (reproducibility assay). The peak areas were determined and compared.
The results for precision tests performed on each of the standard solutions of atenolol (5 μg/mL) showed that the intra-day precision of the method, expressed as RSD (%) from replicate (n = 6) analysis of the same standard solution, was satisfactory (RSD = 0.5 at 10 μg/mL). The inter-day precision (n = 6) was 1.2 at the same concentration (%).
The samples for accuracy were prepared by spiking with the drug (atenolol) at three different levels (lower, medium and upper concentration) – 9, 10, 12 μg/mL – these samples being processed similarly to the calibration samples. Accuracy was calculated as percentage deviation from the spike concentrations.
The accuracy of the method was evaluated by recovery studies analyzing samples spiked with known quantities of drugs and essentially quantitative recoveries were obtained (98.9 ± 0.32).

2.2. Thermal stability studies

The degradation of atenolol was studied in syrup under diferent temperature and storage conditions.
The liquid formulation (syrup) of 10 mg/mL was prepared by addition of atenolol to simple syrup. The formulation was poured into a 30 mL flask. Six vials (three amber and three transparent) were distributed equally to one of three storage conditions: (1) refrigerated (4 °C); (2) room temperature (25 °C) and (3) high temperature (45 °C). After a visual inspection, a 500 μL aliquot was removed from each vial and assayed in triplicate for atenolol concentration on days 0, 7, 11, 21, 28 and 60 by HPLC. The order of the degradation rate was evaluated by the Arrhenius method, using the means of the equations in Table 1.

Table 1: Kinetic equations for evaluated drug degradation

	T_{90}	K
Zero order reaction	0.1. Co/K	Co-C/t
1st order reaction	0.106/K	2.303/t × log Co/C
2nd order reaction	1/9Kx Co	1/t × (1/C – 1/Co)

Table 2: Percentage of the initial concentration of atenolol remaining after storage at different temperatures for 60 days

Time/ formulations	4 °C amber	4 °C transparent	25 °C amber	25 °C transparent	45 °C amber	45 °C transparent
Time zero	100*	100	100	100	100	100
7 days	92.5	92.27	91.43	89.61	85.88	85.37
11 days	83.82	84.52	84.7	84.7	74.45	71.39
21 days	80.96	72.46	79.43	85.88	73.07	69.63
28 days	79.52	69.78	79.13	84.35	62.79	64.32
60 days	71.52	67.53	68.85	66.8	51.36	55.43

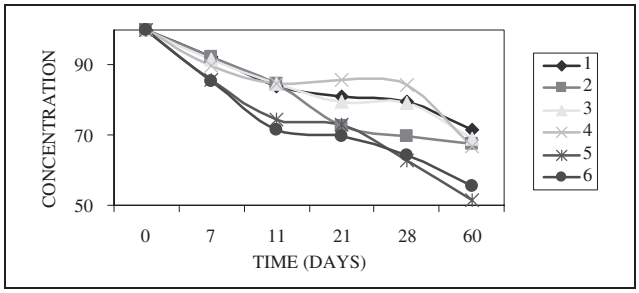

Fig.: Thermal stability of atenolol syrup. 1: Storage at 4 °C in amber flask, 2: Storage at 4 °C in transparent flask, 3: storage at 25 °C in amber flask, 4: storage at 25 °C in transparent flask, 5: storage at 45 °C in amber flask, 6: storage at 45 °C transparent flask

2.3. High performance liquid chromatography analysis

According to recommendations for preliminary degradation studies (ICH 1996), the degradation rate of atenolol was evaluated in a syrup formulation.
The retention time for atenolol was 1.92 min. Under these chromatographic conditions resolution of all the degradation products was achieved in a short analysis time with good peak symmetry.
The initial concentration of atenolol in the samples (day 0) was designated 100%. Concentrations measured on subsequent days were expressed as a percentage of the initial concentration. Stability was defined as the retention of at least 90% of the original concentration. The data in Table 2 show that atenolol is stable in syrup for nine days when stored at 4 °C. Samples gave a mean concentration of drug greater than 92.5% of the initial concentration in this formulation. The degradation increased as a function of time and temperature. The listed values were calculated by comparing the peak area of atenolol to that of the initial concentration (zero time).
A second order model according to Arrhenius described atenolol syrup stored at different temperatures. Linear plots of the inverse of remaining drug concentration against time (days) were obtained corresponding to apparent second-order kinetics. From the slopes of the plots kinetic parameters were estimated (Table 3). Although these kinetic parameters depend on the experimental conditions (temperature, storage), their relative values are of practical utility, suggesting appropriate storage conditions.
The formulation studied was stable in syrup for nine days when stored at 4 °C in an amber vial as shown in the Fig.

3. Experimental

3.1. Chemicals and reagents

Atenolol raw material (RM) was provided by Purifarma® and purified water (HPLC grade) was purchased from Milique®. Methanol and acetonitrile were obtained from Vetec®, and dibasic potassium phosphate and sodium hydroxide (1 N) were obtained from Nuclear®. The saccharose was obtained from União®.

3.2. Apparatus

All HPLC analyses were performed on a Shimadzu chromatographic system equipped with an LC-10AD pump, an UV detector, and a SCL-10AVP

Table 3: Kinetic parameters of atenolol degradation studies

	4 °C amber	4 °C transparent	25 °C amber	25 °C transparent	45 °C amber	45 °C transparent
Kinetic constant (k)	1.16×10^{-4}	1.20×10^{-4}	1.34×10^{-4}	1.66×10^{-4}	2.35×10^{-4}	2.45×10^{-4}
$T_{90\%}$	9.6 days	9.26 days	8.29 days	6.69 days	4.73 days	4.53 days

controller unit. The drug analysis data were acquired and processed with CLASS-VP software used for forced degradation and peak purity determination. The HPLC method employed a C_{18} cartridge (5 μm particle size, 4.6 mm i.d × 150 mm length). A Metachem Technologies Inc. pH meter was used for pH determination.

The mobile phase was 0.01 M phosphate buffer (pH 3.00), methanol and acetonitrile (70 : 15 : 15 v/v/v), delivered at a flow-rate of 1.0 mL/min by an isocratic system. Injection volume was 20 μL. UV detection of atenolol was at 225 nm.

The parameters used in the validation process were selectivity, linearity and range, recovery, precision and limit of quantification.

3.3. Preparation of formulation samples

The atenolol syrup for oral administration was obtained as follows:

A liquid formulation of 10 mg/mL atenolol was prepared from powder and dissolved in about 5 mL of deionized water; filled to volume (30 mL) with simple syrup and transferred to an amber bottle.

3.4. Sample preparation for assay

A sample (5 mg) of raw material was introduced into a 50 mL volumetric flask and diluted with HPLC grade water to yield a theoretical atenolol concentration of 100 μg/mL. Then 500 μL was further diluted to 10 mL to produce a final atenolol concentration of 5 μg/mL to inject into HPLC.

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