Pilot study of the short-term physico-chemical stability of atenolol tablets stored in a multi-compartment compliance aid

Kenneth Chan¹, Julian Swinden, PhD¹, Parastou Donyai, PhD, MRPharmS¹

ABSTRACT
Study objectives: There is a possibility that lower air, moisture and light protection could impact on physico-chemical stability of medicines inside multi-compartment compliance aids (MCCAs), although this has not yet been proved. The objectives of the study were to examine the physico-chemical stability of atenolol tablets stored in a compliance aid at room temperature, and at elevated temperature and humidity to simulate practice conditions.

Methods: Atenolol 100 mg tablets in 28-chamber, plastic compliance aids with transparent lids were stored for four weeks at room temperature and at 40°C with 75% relative humidity. Tablets were also stored at room temperature in original packaging and Petri dishes. Physical tests were conducted to standards as laid down in the British Pharmacopoeia 2005, and dissolution to those of the United States Pharmacopoeia volume 24. Chemical stability was assessed by a validated high-performance liquid chromatography (HPLC) method.

Results: Tablets at room temperature in original packaging, in compliance aids and Petri dishes remained the same in appearance and passed physico-chemical tests. Tablets exposed to 40°C with 75% relative humidity in compliance aids passed tests for uniformity of weight, friability and chemical stability but became pale and moist, softer (82 newtons ± 4; p< 0.0001) than tablets in the original packaging (118 newtons ± 6), more friable (0.14% loss of mass) compared with other tablets (0.005%), and failed the tests for disintegration (>15 minutes) and dissolution (only 15% atenolol released at 30 minutes).

Conclusion: Although chemical stability was unaffected, storage in compliance aids at 40°C with 75% relative humidity softened atenolol tablets, prolonged disintegration time and hindered dissolution which could significantly reduce bioavailability. This formulation could be suitable for storage in compliance aids at 25°C, but not in hotter, humid weather.

Keywords
Drug stability, atenolol, drug packaging, patient compliance, high-performance liquid chromatography (HPLC)

INTRODUCTION
When patients are prescribed medicines there is a general assumption that they will take the full course as prescribed. However, half of all medicines prescribed for patients with long-term conditions are not taken as intended by the pre-

Contact for correspondence: Dr Parastou Donyai
Senior Lecturer in Applied Pharmacy
Department of Pharmacy
Kingston University
Kingston upon Thames
Surrey KT1 2EE, UK
Tel: +44 20 8547 2000 ext 62465
Fax: +44 20 8547 7562
p.donyai@kingston.ac.uk

¹School of Pharmacy and Chemistry, Kingston University, Surrey, UK

Received: 9 March 2007; revised manuscript received: 7 May 2007; accepted 7 May 2007

This non-compliance (or non-adherence) can be intentional (e.g. related to the patient’s beliefs about the medicine) or unintentional (e.g. related to practicalities of medicine taking) [2]. To tackle unintentional non-compliance, pharmacists in primary and secondary care have used multi-compartment compliance aids for dispensing solid-dose medicines. A survey of pharmacies in one region of the UK in 1999 revealed that as many as 77% supplied medication within MCCAs to patients living in their own homes, with a total number of 1,327 patients involved [3]. A new community pharmacy contractual framework in the UK now places emphasis on assessing and providing practical compliance aids to all patients who fall within the protection of the Disability Discrimination Act 1995 and need help with medicine taking [4].

The rationale with most MCCAs is as follows: one compartment corresponds to a single administration time-point and all of a patient’s solid-dose medicines prescribed for that time-point are dispensed into that compartment by pharma-
Physico-chemical stability of atenolol in multi-compartment compliance aids
Parastou Donyai et al

Very little is known about the stability of solid-dose medicines taken out of their sealed, manufacturers' packs and placed together, or even singly, within MCCAs, because manufacturers usually test the stability of their products in the final packaging. This is unusual for pharmacy where other practices that can compromise product stability are increasingly supported by scientific studies. A fitting example is the extensive research that informs the reconstitution, storage and use of parenteral formulations [7]. In relation to MCCAs, it has been suggested that limited available space in each compartment, the fact that MCCAs are not airtight, and lower protection against moisture and light compared with a product's original packaging could all impact on the physico-chemical stability of the medicines stored inside [8].

There are published data on the stability of solid-dose pharmaceuticals per se [9, 10], but none have examined the effect of short-term storage in MCCAs on product stability. Atenolol tablets were selected for this pilot study because they are commonly supplied in MCCAs but manufacturers do not recommend storing these tablets in compliance aids, because of the lack of stability data [8]. The aim was to evaluate the physico-chemical stability of this frequently-prescribed oral solid dosage form when stored in MCCAs for four weeks. The four-week study period is in line with the BMA recommendation to issue 28-day prescriptions for MCCA dispensing [11].

**Methods**

**Storage of atenolol tablets**

Commercially-available tablets of atenolol (100 mg atenolol tablets, Wockhardt UK Ltd, Wrexham, UK, batch number 4L23LD, expiry 09/2006) were used throughout. The study was conducted in June 2006. Atenolol tablets were stored in their original blister-packing at ambient conditions as a control (condition A). Atenolol tablets were also dispensed into one brand of 28-chamber, plastic MCCAs with transparent lids and stored for four weeks at ambient temperature (condition B), and in MCCAs at 40°C with 75% relative humidity (RH) (condition C). Tablets were also stored for four weeks in Petri dishes under ambient conditions (condition D).

**Physical stability study**

The physical stability of atenolol tablets was assessed four weeks after storage to British Pharmacopoeia standards, unless otherwise stated, according to the following methods.

**Visual inspection of appearance**

This was assessed under standard laboratory lighting. Each of 20 tablets per condition was examined for colour and appearance.

**Uniformity of weight**

Each of 20 tablets per condition was weighed separately on an analytical balance, accurate to five decimal places, and the mass recorded. The weight increase or decrease in relation to that of tablets stored under condition A were recorded as a percentage and expressed as the mean of 20 tablets.

**Disintegration test**

Each of six tablets per condition was placed separately in the six cylinders of the disintegration apparatus (Pharma Test Dist 3, serial number 12553), with water (37°C) as the medium. Disintegration was recorded by the operator as the time point corresponding to the breakdown of all six tablets.

**Hardness test**

Each of 10 tablets per condition was placed in turn on the platform of an automatic hardness tester (Static, lot 1194, Schlenninger-4M). The apparatus recorded the hardness in newtons as a measure of the force required to fragment each tablet.

**Friability test**

The total mass of 10 tablets was determined before and after placing them in the clean drum of a friability tester (Pharma Test lot 1193) operated at 25 rotations per minute for four minutes.

**Dissolution test**

The United States Pharmacopoeia 24 (USP 24) specification for dissolution testing of atenolol tablets was modified for use. The dissolution medium, 900 mL de-ionised water thermostated to 37.0 ± 0.5°C, was placed in each of six
vessels of a Varian multi-bath Type II dissolution apparatus with paddle speeds of 50 rotations per minute (rpm). The dissolution medium was sampled at t = 5, 15 and 30 minutes. The samples were filtered with Whatman No. 1 filter paper (filter pore size 0.45 μm) and assayed by using the HPLC protocol described below. Dissolution was deemed acceptable where the concentration of atenolol released at 30 minutes was ≥70% of the total labelled content.

**HPLC assay**

Equipment, conditions and methods of the HPLC assay are discussed below.

**Chromatographic equipment and condition**

The HPLC assay was developed by adapting two published methods [12, 13]. Separation was carried out on a Varian ProStar HPLC with UV detection at 226 nm on a 4.6 x 150mm Spherisorb ODSB column. The mobile phase used was 1 mM ammonium acetate and 2 mM sodium octanesulphonate in a mixture of 25:75 (vol/vol) acetonitrile:water adjusted to pH 3.5 with HPLC-grade glacial acetic acid. The flow rate was 1 mL/minute and the temperature was ambient. The water used to prepare aqueous buffers and dilutions was de-ionised and purified by an Elga Puretab water purification system. The reference standard was pharmacopeial grade atenolol powder (BUFA Pharmaceutical Products, batch number 17 4105, expiry 05/10, Uitgeest, the Netherlands). The internal standard was p-hydroxybenzoic acid (BDH Chemicals Ltd, Poole, UK, batch number 5460630J). All the others reagents used were analytical grade. Injections into the chromatograph were 10 μL each. The assays were performed at least in duplicate.

**Validation of the HPLC method**

The assay was validated for linearity of analytical response, precision of the system, recovery of atenolol, precision of method, and stability-indication.

**Extraction of atenolol from the tablet formulation**

The extraction of atenolol (aqueous solubility of 26.5 mg/mL at 37°C) from the tablet formulation was investigated by a variety of methods that included tablet grinding, sonication, and/or gentle heating. The following method resulted in maximal extraction of atenolol from the formulation and was validated and used for the HPLC assay. Atenolol 100 mg tablets, in quantities of one, two, three or four, were placed in 500-mL volumetric flasks. The internal standard p-hydroxybenzoic acid (5mL of 0.25% w/v solution) was added to each flask and the volume in each made up to the mark with water to produce atenolol concentrations 0.02% w/v, 0.04% w/v, 0.06% w/v, and 0.08% w/v, respectively. Each flask was heated gently in a water bath at 45°C for 30 minutes, and once cooled to room temperature, sonicated for three minutes and the extract solution filtered (Whatman No. 1 filter paper, filter pore size 0.45 μm). The solution extracts were each diluted four-fold to produce final atenolol concentrations of 0.005% w/v, 0.01% w/v, 0.015% w/v, and 0.02% w/v, respectively, before injection into the HPLC system.

**Linearity of analytical response**

A graph of peak area against concentration for a series of standard atenolol solutions with duplicate injections shows linearity of response for the range 0 to 0.025% w/v (regression coefficient 0.9999).

**Recovery of atenolol from the tablets**

A graph of peak area against concentration for a series of solutions containing atenolol recovered from the tablet formulation with duplicate injections for the range 0 to 0.02% w/v also shows linearity of response (regression coefficient 0.9997). Against a labelled claim of 100 mg atenolol per tablet, the mean percentage recovery was 90.4% (± 2.8% standard deviation). Peak areas for atenolol recovered from the tablet formulation displayed a direct linear relationship with the corresponding peak areas for standard atenolol solutions (regression coefficient 0.9995).

**Calibration curve**

A calibration curve was constructed by plotting the response obtained (ratio of area under atenolol peak vs. area under internal standard peak) against atenolol extract concentration with duplicate injections for the range 0 to 0.02% w/v. The calibration curve was subjected to linear least-square regression analysis and found to be linear with a regression coefficient of 0.9911.

**Precision of method**

To determine intra-day precision, 10 samples of atenolol extract standard solutions (0.005%) were prepared and each injected onto the HPLC system. From the mean of these values, the coefficient of variation (CV) was calculated (n = 10) as 6.0%.

To determine inter-day precision, atenolol extract solution (0.005% w/v) was prepared on each of four successive days and injected onto the HPLC system in duplicate. From the mean of each duplicate, the CV was calculated (n = 4) as 2.47%.

**Peak purity**

Peak purity was examined by scanning the chromatographic
The peak of atenolol extracts at wavelengths 226nm and 236nm at concentrations 0.005% w/v and 0.025% w/v. The ratios of the areas under each of these two peaks at both concentrations were within 3% of each other which was deemed acceptable for demonstrating peak purity.

### Table 1: The effect of exposure to extreme pH, heating and freezing on the chemical stability of atenolol as measured by HPLC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Retention time (minutes)</th>
<th>Area under atenolol peak (mAU.min)</th>
<th>Retention time (minutes)</th>
<th>Area under atenolol peak (mAU.min)</th>
<th>Ratio of atenolol peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.47</td>
<td>62.3</td>
<td>7.27</td>
<td>8.8</td>
<td>708</td>
</tr>
<tr>
<td>Freezing (-20°C)</td>
<td>7.44</td>
<td>63.4</td>
<td>7.27</td>
<td>8.7</td>
<td>729</td>
</tr>
<tr>
<td>Heat (65°C)</td>
<td>7.23</td>
<td>63.2</td>
<td>7.23</td>
<td>8.6</td>
<td>735</td>
</tr>
<tr>
<td>pH 12</td>
<td>7.13</td>
<td>49.6</td>
<td>7.13</td>
<td>6.9</td>
<td>719</td>
</tr>
<tr>
<td>pH 12.3</td>
<td>7.19</td>
<td>52.7</td>
<td>7.22</td>
<td>7.3</td>
<td>722</td>
</tr>
</tbody>
</table>

#### Stability-indicating method

A stability indicating study was performed using forced degradation to determine whether atenolol could be distinguished from any degradation products in terms of retention time. Table 1 reveals the effect of forcibly degrading atenolol.

![HPLC chromatograms of atenolol extracted from tablets stored under condition A (original blister-packing at ambient conditions) and condition B (plastic MCCA at ambient conditions)](image)

- **Condition A**
  - Atenolol peak
  - p-Hydroxybenzoic acid

- **Condition B**
  - Atenolol peak
  - p-Hydroxybenzoic acid
Physico-chemical stability of atenolol in multi-compartment compliance aids
Parastou Donyai et al

The European Journal of Hospital Pharmacy Science

by extreme pH (1 and 12, adjusted with 1M hydrochloric acid and 1M sodium hydroxide, respectively), heating at 65°C (for three hours) and by freezing (16 hours). Atenolol stored in the refrigerator (0.01% w/v) was used as a control. In all cases there was a distinction of drug and product peak. The peak purity of the atenolol analyte peak was confirmed by measuring the peak areas at both 226nm and 274nm (>0.98) (CV 1.4%). Atenolol stability was affected only by exposure to extreme acidic and alkaline pH.

Acceptance criteria: chemical stability
The assay of drug concentration was accepted where the concentration of atenolol for each condition was ≥95.0% of the concentration of atenolol stored in blister packaging at ambient temperature measured by HPLC. This is in line with the International Conference of Harmonisation definition of ‘significant change’ for a drug product as a 5% change in assay from its initial value in stability testing [14]. Tablets exposed to 40°C with 75% RH in compliance aids passed the tests for uniformity of weight, friability and chemical stability at week 4 but became paler and moist in appearance, significantly softer (82 newtons ± 4; p<0.0001) than tablets in original packaging (118 newtons ± 6), more friable (0.14% loss of mass) compared with other tablets (0.005%), and failed the tests for disintegration (>15 minutes) and dissolution (only 15% atenolol released at 30 minutes).

RESULTS
The physical and chemical stability of atenolol tablets stored for four weeks under different conditions was determined (Table 2). The temperature in the laboratory remained at 25°C ± 5°C throughout the study period.

Table 2: Physical and chemical stability of atenolol 100 mg tablets stored for four weeks in original blister packaging at ambient temperature (condition A), in MCCAs at ambient temperature (condition B), in MCCAs at 40°C/75% RH (condition C) and in Petri dishes at ambient temperature (condition D)

<table>
<thead>
<tr>
<th>% weight difference compared with condition A</th>
<th>Hardness (N) (± SD) (p-value compared with condition A)</th>
<th>Friability (% difference in mass) (g)</th>
<th>Appearance</th>
<th>Disintegration time (m, s)</th>
<th>Dissolution test (% release at 30 minutes)</th>
<th>Assay of strength (mg) (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets stored in original blister packaging at ambient temperature - Condition A</td>
<td>118 N (± 6)</td>
<td>0.005% - pass</td>
<td>Red tablets, smooth, dry surface</td>
<td>9 m, 27s - pass</td>
<td>80% - pass</td>
<td>108.18 mg (± 8.75)</td>
</tr>
<tr>
<td>Tablets stored in MCCAs at ambient temperature - Condition B</td>
<td>107 N (± 2) (p&lt; 0.0001)</td>
<td>0.009% - pass</td>
<td>Red tablets, smooth, dry surface</td>
<td>7 m 15s - pass</td>
<td>82% - pass</td>
<td>105.62 mg (± 5.92)</td>
</tr>
<tr>
<td>Tablets stored in MCCAs at 40°C/75% RH - Condition C</td>
<td>1.45%</td>
<td>0.14% - pass</td>
<td>Orange tablets, moist appearance</td>
<td>&gt; 15 m - fail</td>
<td>15% - fail</td>
<td>1274.0 mg (± 17.27)</td>
</tr>
<tr>
<td>Tablets stored in Petri dishes at ambient temperature - Condition D</td>
<td>0.98%</td>
<td>0.0002% - pass</td>
<td>Red tablets, smooth, dry surface</td>
<td>6 m 5 s - pass</td>
<td>84% - pass</td>
<td>1279.4 mg (± 6.22)</td>
</tr>
</tbody>
</table>

Key: MCCAs: multi-compartment compliance aids, RH: relative humidity, N: newtons, SD: standard deviation, g: grams, m: minutes, s: seconds

Tablets stored at room temperature in original packaging (condition A), in the MCCAs (condition B) and in Petri dishes (condition D) were similar in appearance at week 4 and passed all physico-chemical tests. However, tablets stored under conditions B and D were significantly softer than tablets stored in original packs (p<0.0001). Tablets exposed to 40°C with 75% RH in compliance aids passed the tests for uniformity of weight, friability and chemical stability at week 4 but became paler and moist in appearance, significantly softer (82 newtons ± 4; p<0.0001) than tablets in original packaging (118 newtons ± 6), more friable (0.14% loss of mass) compared with other tablets (0.005%), and failed the tests for disintegration (>15 minutes) and dissolution (only 15% atenolol released at 30 minutes).

DISCUSSION
Short-term storage of a generic brand of atenolol tablets in plastic multi-compartment compliance aids at ambient temperature did not affect the physico-chemical stability of the tablets compared with that of the same batch kept in original packaging under ambient temperature. However, tablets in MCCAs were shown statistically to be significantly softer than those in original packaging, similar to tablets kept in Petri dishes under the same laboratory conditions. When the same batch of tablets was stored in the same brand of MCCA at 40°C with 75% RH, chemical stability of atenolol was unaffected as were some indicators of physical stability, namely the uniformity of weight and friability. However, these
tablets also became significantly softer, and, more importantly, failed the tests for disintegration and dissolution.

The short-term stability data for atenolol tablets kept in MCCAs at ambient temperature supports the practice of dispensing this solid-dose medicine in similar compliance packaging under typical conditions. However, the prolonged disintegration time and hindered dissolution of tablets kept in MCCAs at 40°C with 75% RH could reduce the bioavailability of the atenolol in these tablets. In addition, tablet appearance changed and this could impact on patient perceptions. While this formulation of atenolol tablets could be suitable for storage in compliance aids at 25°C for four weeks, it does not seem suitable for such storage in hotter, humid weather as experienced in some parts of Europe in the summer months and in tropical climates.

There is a shortage of published scientific papers that examine the physico-chemical stability of other solid-dose preparations in MCCAs. A UK investigation of information on the stability of 392 solid-dose preparations in MCCAs found that none of the products were supported with stability data regarding storage in MCCAs [8]. Instead, the paper provided stability codes for storage of drugs in MCCAs based on information offered by the product manufacturers. The code assigned to the two tablet preparations of atenolol included in that study was “No stability data available, therefore company does not recommend putting in compliance aid. (Refer to SPC [summary of product characteristics] for additional stability information.)”. Although the current study provides some evidence to support the storage of atenolol tablets in MCCAs at 25°C, at least for four weeks, it does not provide data to support the storage of other solid-dose atenolol preparations in MCCAs or storage of the current formulation outside of temperate conditions.

Where applicable, all physical testing was conducted to British Pharmacopoeia standards, except for the dissolution test which was modified for use from USP 24. The modification concerned the quantification of atenolol by HPLC rather than UV spectroscopy because the latter was found to be sufficiently reliable. For the chemical assay, a procedure for extracting atenolol from the intact tablets was developed and fully validated in-house. This method may not be applicable to the extraction of atenolol from other generic or branded tablets as it could be specific to the current formulation only.

The assay values for tablets stored in MCCAs at 40°C with 75% RH and tablets stored in Petri dishes were found to be higher than expected. It is possible that under these conditions a change in the drug release profile of the tablets on storage affected the recovery of the atenolol, creating an artefact. Atenolol is only sparingly soluble in water. Although the use of an organic solvent to extract atenolol from the formulation was investigated, it gave a less linear assay response compared with water. Future studies could look to optimise the extraction process further.

The current study examined storage of one brand of atenolol tablets in one brand of MCCA although several other brands of both atenolol tablets and MCCAs are available on the UK market. The degree of protection afforded (or not) by the current storage device against the degradation effects of light, air, heat and moisture are possibly not the same with other MCCAs. In addition, there was only one sampling time point in this study, at week 4. Further studies should include multiple time points up to the eight-week limit for storage of products in MCCAs, at least as previously set by the RPSGB [6].

This study, on the one hand, supports the storage of a specific formulation of atenolol in one brand of MCCA under ambient conditions for four weeks. On the other hand, because of the potentially significant impact on the bioavailability of atenolol stored at elevated temperature and humidity, this study supports the generally prudent approach taken by manufacturers regarding the storage of solid-dose medicines outside of original packaging in the absence of specific physico-chemical stability data. However, tablets and capsules are routinely dispensed and stored in MCCAs. In the absence of a requirement by regulatory authorities for manufacturers to test the physico-chemical stability of their products outside of original packaging, in MCCAs, more elaborate and specific scientific studies are required to validate this common practice. Further studies could examine the impact of multiple factors such as drug formulation, storage condition, MCCA design, storage period, and co-storage of preparations.

**Conclusion**

Although chemical stability was unaffected, storage in compliance aids at 40°C with 75% RH softened atenolol tablets, prolonged disintegration time and hindered dissolution, which together could significantly reduce bioavailability. This formulation of atenolol tablets could be suitable for storage in compliance aids at 25°C, but not in hotter, humid weather.

**Acknowledgment**

This work was supported by a Vacation Scholarship from the Wellcome Trust.
REFERENCES