Stability of Reconstituted and Diluted Mitomycin C Solutions in Polypropylene Syringes and Glass Vials

Abstract

Purpose: Mitomycin C (MMC) is widely used in treatment of non-muscle invasive bladder cancer at a 1 mg/mL concentration, by intravesical instillation. MMC is also used as an ophthalmic procedure in glaucoma care mostly with 0.2 mg/mL concentration. To accelerate syringes provision, it could be interesting to demonstrate the stability of the drug, in order to be able to prepare the chemotherapeutic drug several hours before the chemotherapy administration.

Methods: A stability indicating HPLC-UV method was developed and validated according to the ICH guidelines. Concentrations of the MMC stored at 25 °C and 60 % of relative humidity and protected from light in polypropylene syringes (1 mg/mL and 0.2 mg/mL) or glass vials (1 mg/mL) were evaluated for 96 h and compared to the initial observed concentrations.

Results: MMC stability was demonstrated in syringes and glass vials at 1 mg/mL only for 8 h in water for injections and for 10 h at 0.2 mg/mL in 0.9 % sodium chloride solutions, because relative concentrations (95 % confidence interval of the mean of 3 samples) were systematically over 90 % of the initial concentrations. After 96 h the relative concentrations were found below 80 % as compared to initial concentrations, thus indicating instability of these solutions. Degradation products were observed and remained below 3 %.

Conclusion: This study confirms that MMC solutions for ophthalmic application at 0.2 mg/mL or vesical instillation at 1 mg/mL have to be formulated extemporaneously to maintain the desired concentration.

Keywords: Mitomycin C, stability, HPLC UV, stability indicating method, ICH

Introduction

Mitomycin C (MMC) is widely used in treatment of non-muscle invasive bladder cancer (NMIBC). In the intravesical instillation indication, according to the Ametycine® product information [1], the MMC concentration required is 1 mg/mL in water for parenteral administration or 0.9 % sodium chloride solution. This solution has to be prepared extemporaneously and an administration kit is delivered by the manufacturer.

Its pharmacological activity is probably due to the quinone, carbamate and aziridine groups that composed the molecule [2]. After a metabolic activation via a reduction, MMC is an alkylating agent that crosslink the DNA. MMC is also used as an ophthalmic procedure in glaucoma care [3]. In fact, the surgical gold standard treatment is the trabeculectomy [4] often associated to MMC in order to prevent subconjunctival scarring and fibrosis. Ophthalmologists generally use a concentration of MMC between 0.2–0.4 mg/mL [5]. The 0.2 mg/mL concentration is the most widely used, according to the American Glaucoma Society [6]. MMC concentration is directly proportional its therapeutic effects [4, 5]. In this indication MMC may be applied to the episclera for a few couples of minutes with the help of microsurgical sponges soaked with the MMC solution provided in syringes by the pharmacy department [4, 5]. In this indication, the activity of the MMC is due to its ability to inhibit the proliferation of the fibroblasts as well as the endothelial cell growth and replication [2].

Hospital pharmacists are, in general, in charge of the preparation of the chemotherapeutic drugs. MMC intravesical instillation is a long procedure with a previous alkalisation of the urine in order to increase its efficacy [7]. Therefore to accelerate the syringe provision in urology wards or ophthalmologic surgery unit, it could be
interesting to demonstrate the stability of the drug, and so to be able to prepare the chemotherapeutic drug several hours before the chemotherapy administration.

MMC is chemically instable in acidic or alkaline conditions as previously demonstrated [8, 9]. In alkaline solutions, the unstable part of MMC is the amine group in the 7 position, replaced by an hydroxyl group [9]. In acidic conditions, the methoxy group is cleaved forming an unsaturated bond and the aziridine ring is opened [8]. The preparation of the MMC for medical uses is performed with water for parenteral administration or 0.9 % sodium chloride solution. The pH of these solutions is near to the neutrality.

To our knowing, there is no MMC stability study of the 1 mg/mL or 0.2 mg/mL in water for parenteral injection or 0.9 % sodium chloride solution in polypropylene syringes.

The aim of this study was to report the stability of MMC at two different concentrations, 0.2 mg/mL in polypropylene syringes and at 1 mg/mL in polypropylene syringes and in glass vial, and to check a possible content-container interaction, as for example a MMC adsorption on the glass or the polypropylene.

Material and method

Reagents

MMC (Ametycine®) 10 mg or 40 mg vials were bought to Sanofi Aventis (Paris, France). All reagents and chemicals were analytical grade and included dihydrogen phosphate potassium, methanol for HPLC (AnalaR Normapur, VWR, Fontenay sous Bois, France), hydrochloric acid (HCl) solution 0.1M (Normadose, VWR, Fontenay sous Bois, France) and sodium hydroxide (Merck, Darmstadt, Germany). Water was obtained from a Prisma reverse osmosis system (Elga, Labwater, Antony, France). Water for parenteral injection was bought to BBraun (Boulogne-Billancourt, France), 0.9 % sodium chloride solution (NaCl) to Aguettant (Lyon, France) and oxygen peroxide (H₂O₂) 10 volumes to Gifrer (Decines, France).

Mitomycin C reconstitution and dilution

MMC was reconstituted according to the manufacturer’s recommendations [1]. Briefly MMC was reconstituted with water for parenteral injection to obtain a 1 mg/mL solution. Half of the solution was transferred into a polypropylene syringe (BBraun, Boulogne-Billancourt, France). The residue was kept in the glass vial. In order to obtain the 0.2 mg/mL concentration, the 1 mg/mL solution was diluted with a sufficient quantity of NaCl 0.9 % and stored in a polypropylene syringe. All syringes and glass vials were protected from light and stored at 25°C and 60 % relative humidity in a climate chamber (Memmert, Schwabach, Germany). For each tested conditions, three containers were prepared and assayed.

Quantification method

HPLC apparatus

MMC concentration of each sample was measured using HPLC with UV detection. The system included a PerkinElmer Series 200 pump, an injector and an oven (PerkinElmer, Waltham, USA). A diode array detector (Flexar PDA detector, PerkinElmer, Waltham, USA) operating between 200–700 nm was used and managed by the Chromera software (V4.10) (PerkinElmer, Waltham, USA). Quantification was carried out at 216 nm. Samples were analysed on a C-18 reversed phase column (Hypersil ODS, 250 × 4.6 mm, 5 µm) from Thermo Scientific, Waltham, USA. The mobile phase was composed of a 10 mM dihydrogen phosphate potassium pH 6.5 buffer (65 %) and methanol (35 %). The flow rate was set to 1 mL/min, the run time was fixed to 12 min. Spectra from 200 to 700 nm were analysed at the apex of the chromatogram peak and compared to the spectrum of freshly reconstituted MMC.

Method parameters

The HPLC method was validated according to the International Conference on Harmonization (ICH Q2 R1) [10]. The linearity of the method was evaluated by three standard curves performed on three different days from 120 to 280 µg/mL (120 µg/mL, 160 µg/mL, 200 µg/mL, 240 µg/mL, 280 µg/mL). The method was considered as linear if the correlation coefficient was over 0.99 for the mean standard curve.

The accuracy of the method was assessed using 9 determinations of three different concentrations (140, 180 and 220 µg/mL) measured three times a day, for three days. The accuracy was measured as the difference between the mean and the accepted true value. Accuracy, for each concentration, has to be less than 5 % to be accepted.
The repeatability was assessed by measuring a 200 µg/mL solution six times. The intermediate precision was evaluated by measuring six times a day for three days this concentration. Repeatability and intermediate precision were determined using the standard deviation of the repeated assays, the threshold value for acceptability was 5%.

**Stability indicating method**

An analytical method is stability indicating if it is able to distinguish the drug from its degradation products. In fact, MMC was submitted to different degradative conditions (alkaline, acidic, light, oxidation, temperature) and the degradation of the MMC was quantified and the degradation products observed.

The alkaline degradation was performed by adding a NaOH solution (0.05 M) in the MMC solution (1:4 v:v) and neutralized after one hour with an HCl (0.05 M) solution. The acidic degradation was carried out by adding a hydrochloric acid solution (0.01 M) in the MMC solution (1:4 v:v) and neutralized after one hour with a NaOH solution (0.01 M). The light degradation was accomplished with a UV-A exposition of 366 nm under a 300 µW/cm² intensity (Chromato-Vue system model CC-20, Ultra Violet Product, Upland, California) during four hours.

Oxidation degradation was performed by adding the H₂O₂ 10 volumes solution (equivalent to 3 % w/w) in MMC solution v:v for one hour.

Finally, temperature degradation was carried out with a MMC solution placed in a lab oven at 90 °C for one hour.

**Determination of MMC concentrations**

Concentrations measured after the reconstitution were considered as the reference 100 %. Next measurements were performed every two hours for 10 h for the 1 mg/mL concentration and after 24, 48 and 72 h and relative concentrations versus reference value were calculated.

For the 0.2 mg/mL syringes, relative concentrations versus reference were measured after two hours and every 24 h for 72 h.

The 1 mg/mL solutions were diluted in water (1:5 v:v).

The relative concentrations were expressed as the 95 % confidence interval (CI 95 %) of the mean. In order to evaluate the influence of the glass or the polypropylene on the MMC storage, a Student’s t-test statistical analysis was performed to compare the relative concentrations for each group. \( P < 0.05 \) was considered statistically significant. The threshold for stability was set to 90 % of the CI 95 % of the mean compared to its initial value without any detectable toxic degradation products.

**pH determinations**

The determination of the pH was performed in triplicate using a HI-122 pH meter (Hanna Instrument, Tannerie, France). It was carried out just after the reconstitution of the drug and every two hours for the 1 mg/mL vials and syringes for 10 h, and every 24 h for 96 h. For the 0.2 mg/mL pH was measured just after the dilution and after two and ten hours and each 24 h for 72 h.

**Visual examination**

A visual examination was performed along the stability study in order to detect a potential precipitate or colour change.

**Results**

**HPLC method parameters**

The retention time of MMC was estimated to 5.50 min. The peak was well defined and symmetric and no impurity was found (Figure 1).

The three standard curves were very similar and the mean equation was: \( y = 3.48x - 1.52 \) where \( x \) was the area under the curve. The correlation coefficient (\( r^2 \)) was greater than 0.999.

The accuracy of the method was estimated to 4.27 %, 4.03 % and 1.94 % respectively on the 140, 180 and 220 µg/mL concentrations. The repeatability of the method was systematically inferior to 5 % (3.49, 1.40 and 2.89 % for the day 1, 2 and 3 respectively) and the intermediate precision was evaluated 2.59 %.

**Stability indicating method**

For each tested conditions, MMC concentrations were systematically inferior to a fresh MMC solution not exposed to the force degradation conditions (Table 1), and a degradation product is observed on the chromatogram (Figure 1).
Relative concentrations and spectra

Relatives MMC concentrations were measured and presented in Table 2. MMC degradation over the time has been observed with a progressive appearance of degradation products.

After 96 h, there is no statistical difference between MMC relative concentrations in glass vial or in polypropylene syringes ($p = 0.99$).

All UV spectra from 200 to 700 nm extracted at the apex of the MMC chromatogram peak were strictly overlaid to the reference solution. This result demonstrated the purity of the peaks thus the absence of a degradation product hidden below the MMC peak.

Degradation products corresponding to the acidic or alkaline stress conditions were observed around 4.10 min run time. The ratio between the area of these products and MMC were systematically below 1.5% at 10 h and reached 3% at the maximum along the study.

**pH determination and visual examination**

The pH of each solution within every storage condition was monitored and presented in the Table 3 from $T = 0$ to the end of the study. An alkalinisation (up to 0.9 pH unit in 96 h) of solutions was observed for each tested conditions. No modification of the visual aspect of the test solutions was observed during the stability study.

**Discussion**

For the analytical method, the correlation coefficient of the mean calibration curve was over than 0.99, so the method was considered as linear. Moreover, the accuracy, the repeatability and the intermediate precision
were systematically inferior to 5%, the method was so considered as accurate, repeatable and with an acceptable intermediate precision.

About the stability indicating capacity of the method, for each tested condition (hydrolysis, oxidation, reduction and photolysis) a degradation peak was observed and the relative concentration of the MMC for the tested conditions were systematically between 56 to 87%. The method is thus considered as accurate, repeatable and with an acceptable intermediate precision.

Table 2: Mitomycin C relative concentrations along time after storage in ICH climatic chamber at 25 °C 60 % relative humidity, expressed as the 95% confidence interval of the mean (for each conditions, three different syringes or glass were tested).

<table>
<thead>
<tr>
<th>Analysis time (h)</th>
<th>Syringe 1 mg/mL</th>
<th>Glass vial 1 mg/mL</th>
<th>Syringe 0.2 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>975.03 ± 10.40</td>
<td>899.70 ± 23.85</td>
<td>192.30 ± 0.99</td>
</tr>
<tr>
<td>2</td>
<td>95.74 ± X &lt; 98.32</td>
<td>92.80 ± X &lt; 107.4</td>
<td>99.57 ± X &lt; 100.8</td>
</tr>
<tr>
<td>4</td>
<td>94.71 ± X &lt; 95.04</td>
<td>97.86 ± X &lt; 105.9</td>
<td>/</td>
</tr>
<tr>
<td>6</td>
<td>92.26 ± X &lt; 94.21</td>
<td>96.21 ± X &lt; 103.0</td>
<td>/</td>
</tr>
<tr>
<td>8</td>
<td>92.08 ± X &lt; 92.46</td>
<td>90.88 ± X &lt; 100.9</td>
<td>/</td>
</tr>
<tr>
<td>10</td>
<td>89.36 ± X &lt; 89.98</td>
<td>88.23 ± X &lt; 95.43</td>
<td>90.55 ± X &lt; 91.63</td>
</tr>
<tr>
<td>24</td>
<td>80.91 ± X &lt; 87.37</td>
<td>81.63 ± X &lt; 90.78</td>
<td>88.11 ± X &lt; 89.16</td>
</tr>
<tr>
<td>48</td>
<td>83.40 ± X &lt; 86.62</td>
<td>81.91 ± X &lt; 88.63</td>
<td>87.69 ± X &lt; 88.74</td>
</tr>
<tr>
<td>72</td>
<td>79.86 ± X &lt; 82.02</td>
<td>73.81 ± X &lt; 84.49</td>
<td>82.09 ± X &lt; 83.07</td>
</tr>
<tr>
<td>96</td>
<td>76.06 ± X &lt; 79.34</td>
<td>74.26 ± X &lt; 81.18</td>
<td>78.00 ± X &lt; 78.93</td>
</tr>
</tbody>
</table>

Note: / not realized.

Table 3: pH of the mitomycin C solutions in different conditions for 96 h (n = 3 for each time point). The pH is expressed as the mean ± standard deviation.

<table>
<thead>
<tr>
<th>Analysis time (h)</th>
<th>Syringe 1 mg/mL</th>
<th>Glass vial 1 mg/mL</th>
<th>Syringe 0.2 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.42 ± 0.03</td>
<td>6.42 ± 0.03</td>
<td>7.13 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>6.59 ± 0.03</td>
<td>6.65 ± 0.01</td>
<td>6.99 ± 0.02</td>
</tr>
<tr>
<td>4</td>
<td>7.01 ± 0.07</td>
<td>6.58 ± 0.08</td>
<td>/</td>
</tr>
<tr>
<td>6</td>
<td>6.82 ± 0.06</td>
<td>6.56 ± 0.06</td>
<td>/</td>
</tr>
<tr>
<td>8</td>
<td>7.18 ± 0.08</td>
<td>6.61 ± 0.11</td>
<td>/</td>
</tr>
<tr>
<td>10</td>
<td>7.02 ± 0.12</td>
<td>6.59 ± 0.03</td>
<td>6.88 ± 0.06</td>
</tr>
<tr>
<td>24</td>
<td>7.23 ± 0.09</td>
<td>6.99 ± 0.04</td>
<td>7.32 ± 0.02</td>
</tr>
<tr>
<td>48</td>
<td>7.24 ± 0.01</td>
<td>7.17 ± 0.04</td>
<td>7.33 ± 0.04</td>
</tr>
<tr>
<td>72</td>
<td>7.19 ± 0.06</td>
<td>7.12 ± 0.00</td>
<td>7.30 ± 0.03</td>
</tr>
<tr>
<td>96</td>
<td>7.32 ± 0.09</td>
<td>7.28 ± 0.04</td>
<td>/</td>
</tr>
</tbody>
</table>

Note: / not performed.

were systematically inferior to 5%, the method was so considered as accurate, repeatable and with an acceptable intermediate precision.

During the drug product conservation such as temperature elevation and oxidation with an air contact in the storage content thus justifying the other stress conditions applied to the forced degradation study.

The relative concentrations of the MMC demonstrated the instability of the MMC in water at 1 mg/mL and in NaCl 0.9% at 0.2 mg/mL after 8 h and 10 h respectively, from 10 h, relative concentrations for each condition were inferior to 90% of the initial concentrations. Because there is not any significant difference between the polypropylene syringes and the glass vial at 1 mg/mL no content-container interaction with these two materials could be demonstrated, that was in accordance with previous paper, at others concentrations [11].

Degradation products were observed along the study corresponding to acidic or alkaline forced degradation.
The peaks observed after oxidation, UV and heat stress were not observed. This can be explained by the fact, that the syringes and glass vials were not exposed to light, did not contain oxygen, and were stored in a climate chamber at 25 °C.

No threshold for acceptance of degradation products was set because the toxicity of the degradation products are unknown and not studied in the literature to our knowing. For this reason, even if these degradation products were found below 1.5% at 10 h of the MMC concentration, it should be advised to use freshly prepared MMC solution.

All UV spectra extracted at the apex of the maximum chromatogram peak were strictly similar to a fresh MMC solution demonstrating that the maximum peak was effectively corresponding to MMC and that the peak was pure. This procedure is a classical identification and purity test.

Concerning the pH measurements, there is a difference between the T = 0 and the end of the study for each condition. The evolution is less important in the 0.2 mg/mL condition than in the 1 mg/mL (for both content conditions). The evolution is not different for the 1 mg/mL concentration either in a polypropylene syringe or in a glass vial. This pH evolution could be relied to the degradation of the MMC already attested by the relative concentrations measurements with the HPLC-UV method.

We demonstrated in this study a very short time conservation of MMC, and this was in contradiction with previous results [11], which demonstrated a stability of MMC up to three months, probably because the pH of the reconstituted medium was adjusted to 7.2. However, previous papers [5, 12] have already postulated a rapid MMC degradation at 0.4 mg/mL over the time at room temperature. At 0.05 mg/mL MMC was not stable over 12 h in room temperature condition or in cold condition (5 °C) [13].

Finally we did not conduct the study at a temperature of 4 °C, which probably could enhance the drug stability, as it was determined previously [11]. However, a drug stability of 8 h could be sufficient to prepare the reconstitution of the drug and to provide wards with no required particular preserving precaution. An efficient organization in the hospital has thus to be developed between pharmacy, transport services and wards.

Microbiological stability of the MMC solutions have not been tested, but it is known that MMC is an antibiotic with a significant antibacterial activity [14], and all of the preparation are made in aseptic conditions in accordance with a quality assurance program including environmental and team monitoring.

**Conclusion**

A HPLC UV stability indicating method was developed and validated according to the ICH to quantify the degradation of the MMC over the time. Two different concentrations were studied, 1 mg/mL used in the treatment of NMIBC, and 0.2 mg/mL in glass vials and polypropylene syringes. After 10 h our study showed that CI 95% of the mean initial concentration fell under 90% while some degradation products in low quantity are observed.

Thus, unfortunately, our results confirmed that MMC solutions for ophthalmic application at 0.2 mg/mL or vesical instillation at 1 mg/mL have to be formulated extemporaneously to maintain the desired concentrations and avoid potential toxic effects. These instabilities require an adequate organization between the pharmacy and wards.

**Conflict of interest statement:** The authors state no conflict of interest. All authors have read the journal’s Publication ethics and publication malpractice statement available at the journal’s website and hereby confirm that they comply with all its parts applicable to the present scientific work.

**References**


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