Preparation Method and Stability of a Temozolomide Suspension: A Pilot Study

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ABSTRACT

Aim: To investigate a compounding process for temozolomide 10 mg/mL suspension from capsules without using a mortar and pestle; and the stability of the suspension under refrigeration (2 to 8 °C) and at room temperature (22 to 23 °C).

Method: Temozolomide 10 mg/mL suspension was prepared using a modified formulation and method to contain the product in the final container and limit operator exposure to cytotoxic powder or residue. Decontamination was undertaken of work and finished product surfaces. Samples were stored at room temperature and under refrigeration. Both samples were protected from light and stored in identical polypropylene containers. High-pressure liquid chromatography was used to assay the potency of the suspension.

Results: The sample refrigerated and protected from light exhibited little or no chemical degradation for 22 days. At room temperature, the concentration fell below the acceptable concentration after 8 days. An acceptable product was produced which re-suspended evenly when shaken.

Conclusion: Preliminary results indicate that temozolomide 10 mg/mL suspension prepared via this method, refrigerated, protected from light and stored in polypropylene containers may be stable for up to 22 days.

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INTRODUCTION

Capsules are the only oral dose form of temozolomide available in Australia, making it difficult to administer a safe and accurate dose to children when dealing with portions of temozolomide capsules. Instructions for parents are available for opening temozolomide capsules into a medicine cup and mixing with apple juice/sauce with the use of personal protective equipment (e.g. mask, goggle, gloves) and containing airborne particles within a clear plastic bag.[1,2] The availability of a ready-to-use liquid dose form would be less of an occupational health and safety risk. The International Society of Oncology Pharmacy Practitioners' (ISOPP) recommends: 'The opening of capsules, crushing tablets, and dissolving powders should not be done outside of the pharmacy. The crushing of tablets and the mixing of powders generates airborne particles of the products used and should be avoided wherever possible. The crushing of cytotoxic tablets or the opening of capsules in an open mortar should also be avoided.'[3] These recommendations must be followed by hospital pharmacists required to compound cytotoxic liquid dose forms from tablets or capsules.

An existing formulation using a mortar and pestle is available for pharmacists to compound temozolomide suspension (Table 1, Appendix 1).[4] Cytotoxic drugs are toxic and exposure can pose significant health hazards, such as mutagenicity, reproductive, teratogenic and genotoxic effects.[5-8]

The existing formulation was modified, so as to contain temozolomide powder liberated from opening capsules (Table 1, Appendix 2). Ideally, capsules should be allowed to disintegrate and disperse in a pre-calibrated bottle. However, there was concern that the gelatine and other ingredients in the capsule shell may affect the stability of the final product.[3] An investigation into the feasibility of this process resulted in an unacceptable product, with the capsule shells disintegrating but not completely dissolving after 48 hours. Dissolving capsules in an oral syringe is often too technical for parents to undertake.

Table 1. Temozolomide 10 mg/mL suspension formulations

Ingredients	Existing formulation[4]	Modified formulation
Temodal 100 mg capsules	10	10
Povidone-K 30	500 mg	500 mg
Citric acid	25 mg (anhydrous)	27.3 mg (monohydrate)
Purified water (water for injection)	1.5 mL	10 mL
Ora-Plus (suspending agent)	50 mL	45 mL
Ora-Sweet (flavouring agent)	100 mL	100 mL

The existing formulation was compounded in a mortar and pestle in a fume cabinet, while the modified formulation was compounded in the final container in two stages (Appendix 2).

This study aimed to investigate a compounding process for temozolomide 10 mg/mL suspension from capsules using a mortar and pestle; and the stability of the suspension under refrigeration (2 to 8 °C) and at room temperature (22 to 23 °C).

METHOD

Temozolomide 10 mg/mL suspension (100 mL) was prepared in two stages (Appendix 2). To aid dispersion of the capsule contents during compounding, the purified water content of the existing formulation was increased, and the amount of Ora-Plus and Ora-Sweet were proportionally decreased (Table 1).[4]

Personal protective equipment was worn during compounding. A control formulation was also prepared containing no temozolomide. Two 20 mL samples were taken – one was stored at room temperature and the other refrigerated for the duration of the study. Both samples were stored in identical polypropylene containers as the bulk suspension.

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The temozolomide concentration was determined using an adapted version of a validated high pressure liquid chromatography (HPLC) method for up to 36 days.[9,10] The HPLC system consisted of a LC1500 pump (ICI Instruments), TC 1900 temperature controller, DP 800 Data Station, Kortec K95 variable wavelength UV detector (316 nm) and a Rheodyne 7125 injector (20 µL sample loop). A Spherisorb ODS2 (C18) column (25 cm x 4.6 mm) set at 30 °C was used.

The mobile phase consisted of aqueous acetic acid 0.1% and acetonitrile (90:10) pumped at a flow rate of 1 mL/min. Analytical stock solution of temozolomide (98% purity, Sigma-Aldrich) was prepared in a dissolving solution, consisting of aqueous methanol 20%, glacial acetic acid 0.1% and hydrochlorothiazide 0.05% (Sigma-Aldrich) as the internal standard. The calibration curve was constructed over the temozolomide concentration range (0.1 to 1 mg/mL) using peak area ratio of temozolomide: hydrochlorothiazide (correlation coefficient = 0.999).

Temozolomide and the internal standard eluted at approximately 4.8 and 9.4 minutes, respectively. Chromatograms of samples prepared from temozolomide capsule (Temodal, Schering-Plough) did not show interfering peaks.

Temozolomide suspension samples were analysed the day after preparation and weekly for 5 weeks. Each sample was analysed in triplicate with a calibration curve.

The container of the sample suspension was shaken vigorously for about 30 seconds and then placed in an ultrasonic bath for 5 minutes. Aliquots (0.5 mL) were taken from each sample and transferred to a 10 mL volumetric flask. Methanol (0.5 mL) was added and further diluted with the dissolving solution to 10 mL. The samples were completely covered with aluminium foil to prevent decomposition of temozolomide by UV light. The resulting samples were subjected to thorough mixing using the ultrasonic bath for a further 5 minutes and 20 µL of this sample was injected for HPLC analysis.

Data are presented as mean and standard deviation.

RESULTS

No caking or clumping of the temozolomide suspension was observed during the duration of the pilot, and the suspension evenly re-suspended when shaken. The samples had a pinkish colour at the start of the analysis. The refrigerated sample maintained this colour for the duration of the pilot (5 weeks), whereas the sample stored at room temperature darkened after the first week and became brown by the end of the pilot. Temozolomide concentration on Days 1, 8, 15, 22, 29 and 36 after preparation for the refrigerated and room temperature samples are presented in Figure 1 and Table 2.

The refrigerated sample exhibited no loss for the first 22 days, but fell below the pharmaceutical acceptable shelf-life concentration of 90% from Days 23 to 29. This is shorter than the shelf-life of 60 days previously reported. At room temperature, loss of temozolomide was rapid with approximately 6% at Day 8, 16% at Day 15, 21% at Day 22, 40% at Day 29 and 55% at Day 36.[4] This is comparable to the results for the first 21 days at 23 °C from the previous report (Table 3).[4] The pH of the samples were tested after 36 days using Acilit pH (0-6) indicator strips (Merck) and gave a pH value of 4.5 for the refrigerated sample and a pH value of 5 to 5.5 for the room temperature sample.

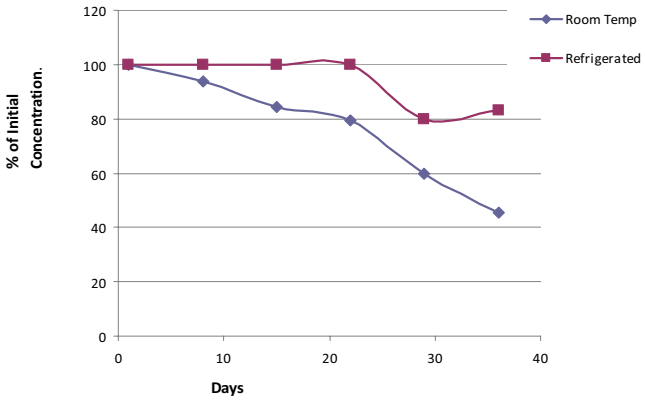

Figure 1. Stability of modified temozolomide 10 mg/mL suspension during refrigeration and at room temperature.

Table 2. Stability of modified temozolomide 10 mg/mL suspension under refrigeration and at room temperature

	Initial concentration remaining*					
Sample	**Day 1**	**Day 8**	**Day 15**	**Day 22**	**Day 29**	**Day 36**
3-4 °C	101±2	108±6.1	104±1.5	108±8.6	81±4	83±2.5
22-23 °C	100±7.1	94±7.1	84±1.5	79±1	60±5.6	45±0.5

*Triplicate determination of each sample; mean ± SD.

Table 3. Previously reported stability of temozolomide 10 mg/mL suspension[4]

	Initial concentration remaining*						
Sample	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 30**	**Day 45**	**Day 60**
4 °C	-	100±1.2	100±1.1	-	99±0.6	101±1.2	99±1.4
23 °C	98±1.4	94±1	86±2.3	79±2.1‡	-	-	-

*Triplicate determinations of triplicate samples except where noted; mean ± SD. †Two samples tested; mean ± SD.

DISCUSSION

Besides 'not to open capsules or crush tablets containing cytotoxic drugs' the SHPA and Worksafe Victoria guidelines do not offer practical advice for pharmacists required to compound oral cytotoxic preparations to overcome compliance or safety issues for special groups.[11,12] The *Safe Handling - Cytotoxic Drugs and Related Waste* suggest: 'not to crush or break tablets or capsules for any reason, e.g. nasogastric or PEG feed-outside the pharmacy's cytotoxic preparation area'.[13] Manufacturers also do not always elucidate how to overcome dosing administration issues in patients with swallowing difficulties, e.g. in paediatrics.

The ISOPP standards give hierarchical directions for protection, such as replacement, isolation of the hazard, source containment, engineering controls/ventilation and the use of personal protective equipment.[3] These standards recommend that oral cytotoxic drugs should be prepared extemporaneously under the same conditions as parenteral cytotoxic drugs, in a room dedicated for this purpose. A class II cytotoxic drug safety cabinet (CCDSC) can be used, but should not be used for mixed activity (sterile and non-sterile) because of the potential for the liberation of powders and other particulate contamination into the clean room and cabinet. The risk of this type of contamination is high.[3] These resources are not readily available to most hospital pharmacists faced with the safe preparation of cytotoxic compounds, routinely necessitating the use of CCDSC and clean

rooms also used to compound sterile products. Controlled preparation with decontamination of work surfaces in conjunction with microbiological environmental monitoring is a dispensation in the absence of designated facilities for the majority of hospital pharmacies. The preparation method described in the present study aims to contain powder liberated from opened capsules within the final container. To offer operator protection during compounding the CCDSC is left on.[3]

As temozolomide is degraded in highly alkaline solutions, it is recommended that decontaminating solutions (pH ≥ 9) be used to wipe over work surfaces (inside of the CCDSC and outside container of the final product).[14,15] This should be performed when compounding is complete to reduce temozolomide surface contamination. Biogram 1% v/v disinfectant (Ecolab) in 100 mL sterile water for irrigation has a pH of 9.5 to 10 and is ideal for this application. This step should be followed by irrigation with sterile water to remove excess Biogram solution, and then sterile alcohol 70%. Routine microbiological monitoring via settle plates and surface swabs should be performed during compounding to verify that microbiological contamination has not been introduced into the CCDSC. Routine surface wipe testing and analysis to detect cytotoxic contamination is not readily available to most hospital pharmacies. The cost involved to undertake extensive and meaningful testing makes it unaffordable and is not routinely performed at the study hospital. The occupational health and safety issues involved in analytical testing of cytotoxic compounds also limits hospital pharmacies from safely performing HPLC testing, requiring the use of qualified external facilities.

Temozolomide is hydrolysed rapidly at neutral and alkaline pH and is stable at acidic pH. To maintain the pH below 5, citric acid is included in the formulation. Povidone K-30 is added to prevent temozolomide crystallisation.[4] Temozolomide suspension must be stored in a plastic container because it leaches out of hydroxide from glass. A wide-mouth polypropylene container was sourced from sterile water for irrigation 100 mL (Baxter) and fitted with a 38 mm child proof 'click-clock' lid (Silverlock packaging). From the preliminary results, the shelf-life is probably 25 days; however a 22-day expiry is assigned at this stage.

Surface contamination from cytotoxics is an issue impacting on Australian hospital pharmacies that compound cytotoxic products.[16-18] The use of a mortar and pestle to compound cytotoxic suspensions requires washing and decontamination when compounding is complete. Standards recommend that equipment should be set aside for cytotoxic drugs (not only one specific drug) and cleaned immediately after use with a strong alkaline solution.[3,11] This does not safe guard against operator exposure during cleaning and product cross-contamination if not cleaned properly. With this manufacturing method and associated cleaning process, contamination of the work environment and operator exposure is likely to occur. Ideally, equipment such as the mortar and pestle should be replaced with a disposable system as described. Source containment should be continuous throughout the manufacturing process.[3] Personal protective equipment must be used when handling or compounding cytotoxic products.

In conclusion, preliminary results indicate that temozolomide 10 mg/mL suspension prepared via this method, refrigerated, protected from light and stored in polypropylene containers may be stable for up to 22 days. However, due to the small sample size this method requires further validation.[19] The effectiveness of the described method in reducing cytotoxic contamination and improving operator safety has not been validated and should be investigated.

Competing interests: None declared

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Appendix 1. Temozolomide 10 mg/mL suspension prepared using a mortar and pestle in a fume cabinet[4]

Wear suitable protective garb during preparation.

1. Empty contents of 10 Temodal 100 mg capsules into a glass mortar.
2. Add 500 mg of povidone K-30 powder.
3. Triturate to mix and reduce the mixture to a fine powder.
4. Dissolve anhydrous citric acid 25 mg in purified water 1.5 mL.
5. Add to the mortar and wet the powder.
6. Mix to form a uniform paste.
7. Add some Ora-Plus and mix into a uniform mixture.
8. Add the balance of the Ora-Plus.
9. Transfer to a graduated glass cylinder.
10. Rinse the mortar and pestle with small aliquots of Ora-Sweet; repeat the rinsing three more times.
11. Add an additional amount of Ora-Sweet to the graduated cylinder to bring the final volume to 100 mL. Shake well.
12. Package in amber plastic prescription bottles and label with 'Shake Well' and 'Refrigerate' and the beyond-use date.

Appendix 2. Temozolomide 10 mg/mL suspension prepared using the modified formulation and method

Stage 1: In the general manufacturing area.

1. Pre-calibrate wide-mouth polypropylene bottle 100 mL (Baxter) to final volume mark. Replace original lid with a 38 mm child-proof 'click-clock' lid (Silverlock packaging).
2. Weigh povidone-K 30 and citric acid monohydrate and transfer into the wide-mouth bottle.
3. Wash weigh trays with purified water and transfer remnant powder into bottle. Add any remaining purified water.
4. Recap and shake well to dissolve powders (keep sealed).

Stage 2: Inside the CDSC and CCR. The CDSC should be left on. Personal protective equipment must be used.

1. Wipe equipment, consumables and sealed ingredients with sterile alcohol 70% and transfer into CCR.
2. Wipe over inside surfaces of CDSC with sterile alcohol 70%. Place down sterile chemo spill mat. Respray consumables with sterile alcohol 70% and allow to dry.
3. Transfer sealed container from Stage 1 into CDSC, having sprayed with sterile alcohol 70%, wiped over with a sterile wipe and allowed to dry.
4. Spray sterile alcohol 70% onto sterile nitrile gloved hands and allow to dry. Open temozolomide 100 mg capsules over the wide-mouth bottle and empty contents into the solution.
5. Replace child-proof lid and shake well to disperse.
6. Allow to stand for 1 minute, remove cap and add required amount of Ora-Plus and then Ora-Sweet to final volume mark.
7. Replace lid and shake well.
8. Measure pH (pH must be < 5).[4]
9. Place contaminated consumables and waste into press seal bags.
10. Dispose of chemo spill mat and replace outer gloves.
11. Decontaminate outer surface of the bottle and inside surfaces of CDSC with Biogram 1% v/v solution (Biogram concentrate 1 mL in 100 mL of sterile water for irrigation).
12. Wipe surfaces with wipe moistened with sterile water for irrigation to remove Biogram solution residue. Wipe inside of the CDSC with sterile alcohol 70%.
13. Label, seal in a clear plastic bag and a labelled light protective bag.
14. Store in refrigerator at 2 to 8 °C.

CCR = cytotoxic clean room. CDSC = cytotoxic drug safety cabinet.