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Rapid communication

Design and stability study of a paediatric oral solution of methotrexate 2 mg/ml

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ABSTRACT

Oral paediatric forms development by pharmaceutical industry is still insufficient. The present study was performed to propose an adapted and pleasant formulation of liquid oral formulation of MTX. The solution is composed of injectable methotrexate, water, Ora Sweet® and sodium bicarbonate.

After 120 days storage, pH remained stable at about 8 in all formulations, insuring no risk of MTX precipitation. MTX content in solution formulation, determined by high performance liquid chromatography measurements, remained in the specifications of >90% of the initial concentration when stored at 4 and 25 °C. Forced degradation of MTX by heat and acidic conditions allowed formation and detection of degradation products by the analytical method.

Microbial study of the preparation shows that the solution remains in the specifications during all the storage, or after one sample each week during one month, eventually indicating the microbial properties are not affected by patient use.

To conclude, we here propose a new MTX liquid formulation stable for at least 120 days.

1. Introduction

Drug delivery to children is still limited by the lack of licensed drugs and non adapted forms for administration (Fontan et al., 2004). Methotrexate (MTX) is widely used in paediatrics, mainly for lymphoid acute leukaemia (LAL) treatment or rheumatoid disease (Weiss et al., 1998; Otten et al., 2002). Despite its use for many years, no liquid oral formulations are marketed. Tablets are not indicated for children younger than 6 years. Thus, most hospital pharmacies have to prepare hard capsules but there are 3 drawbacks: (i) the lack of possibility to adapt doses once the capsules are prepared, i.e., in case of toxicity, dose can be reduced, or on the contrary a good tolerance leads to doses enhancement, (ii) drug possible bad taste (bitterness..) and (iii) family exposure to cytotoxic at home when the capsules are opened for administration. Lack of oral liquid forms also leads to use of injectable medicines for oral administration. Such a practice poses the problem of pH incompatibility, or non adapted ingredients for oral use. For these reasons, oral solutions or suspensions are emerging in hospital pharmacies. For instance spironolactone, hydrochlorothiazide and captopril (Fajolle et al., 2005), sildenafil (Provenza et al., 2014), ursodeoxycholic acid (Santovena et al., 2014), or even reconstitutable hydrocortisone (Orlu-Gul et al., 2013) formulations are described.

In the present study, in order to develop a new formulation, we focused on MTX physicochemical properties: MTX is poorly soluble in water and alcohol. MTX precipitates at pH less than 6.6 (Allwood et al., 2002). Furthermore, MTX degrades when exposed to light. In 1979, a formulation of syrup containing sodium bicarbonate, injection MTX, simple syrup and chloroform water was proposed, and was shown to be stable for period of up to 1 month (Stuart et al., 1979). Currently, chloroform water is no more suitable as it is toxic. Recently, another team prepared MTX solution in Ora Plus® and Ora Sweet® (Megias Vericat et al., 2012). The stability was shown to be 25 days, which is insufficient for an optimal patient comfort and for a pre-empted fabrication. Thus, our hospital developed a new oral formulation. The present work studies organoleptic characteristics and chemical stability of MTX oral formulation. As MTX is likely to be given to immunocompromised children, microbial study was also conduced to insure a satisfying microbial quality.

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2. Material and methods

2.1. Reagents

The oral solution was performed with injectable MTX 500 mg/20 ml (Mylan, Pittsburg, USA), sodium bicarbonate (COOPER, Melun, France), OraSweet® (Paddock laboratories, Minneapolis, USA) and injectable water (Aguettant, Lyon, France). All ingredients were Pharmacopeia grade.

For HPLC, acetonitrile (Hipersolv Chromanorm, VWR, Fontenay sous Bois France), potassium hydroxyde and potassium dihydrogen phosphate (ACROS Organics, Geel, Belgique) were used. Water was obtained from a Prima reverse osmosis system (Elga Labwater, Antony, France).

All reagents and solvents were analytical grade.

2.2. Storage

MTX solution was packaged in 30 ml amber type I glass bottle, for 120 days, in triplicate. Storage was performed in climatic chamber qualified according to ICH at 4 °C and 25 °C under 60% relative humidity.

2.3. Forced degradation

MTX solution was incubated with sulphuric acid 0.5 M for 60 min at 70 °C ($n = 3$).

2.4. Chromatographic conditions

A high pressure liquid chromatography (HPLC) method was developed. The system was characterized by a PerkinElmer Series 200 pump, injector and oven. The detector was a diode array detector (Flexar PDA detector, PerkinElmer, Walthman, USA) operating between 190 and 700 nm. Chromera software (v4.1.0) (Perkin0Elmer, Walthman, USA) was used to quantify the peaks of the chromatograms. The mobile phase consisted of a mixture of sodium dihydrogen phosphate buffer which pH is adjusted to pH 6.6 and acetonitril (90:10 v:v). The flow rate was set to 1 ml/min. A C18 ODS Hypersil (250 mm x 4.6 mm, 5 μm) (Thermo-Scientific, Villebon sur Yvette, France) was used and maintained at 20 °C. The sample injection volume was 10 μL and the analysis time was 10 minutes. MTX detection and quantification were processed at 302 nm.

2.5. Method validation

2.5.1. Method validation was performed according to ICHQ2

MTX content in solution was determined by diluting 500 μl sample with 9.5 ml of mobile phase. In order to remove Ora Sweet®, 5 ml of the diluted mix were centrifuged for 7 min at 4000 rpm. 100 μl of supernatant were then diluted with 900 μl of mobile phase.

Working standard solutions for the calibration curves were prepared using same ingredients as in formulation, to reach a final MTX concentration of 1, 1.5, 2, 2.5 and 3 mg/ml. Then these working standard solutions underwent same dilution as sample formulation, e.g., in mobile phase leading to concentrations between 5 and 15 μg/ml.

2.6. Organoleptic appreciation

Odor, appearance and color of the preparation were assessed. Because of the intrinsic toxic properties of MTX solution, taste and palatability were not studied.

2.7. pH study

Measurements were performed in triplicate using a pH meter Consort C561 (Tunhout, Belgium).

2.8. Stability study

MTX formulations are considered stable if physical characteristics have not moved and if drug concentration remains above 90% of the initial concentration without absence of characteristic degradation peaks.

2.9. Microbiological study

Three conditions (each in triplicate) were studied (Table 1):

- Sample days same as patient taking medication at home, e.g., days 7, 14, 21 and 28.
- Analyse after 120 days, corresponding to the physico chemical stability of the solution.
- Positive control: the solution containing or not MTX is artificially infected with environment bacteria at day 0 and analysed after 7 days. Infected MTX solutions were used as controls to insure MTX did not inhibit microbiological growing.

To perform analysis, each sample was diluted 1:10 in sterile NaCl 0.9%, and then plated on different media and different conditions:

- Sheep blood agar plate (Thermo Fisher Scientific, United Kingdom), aerobic and anaerobic incubation at 37 °C
- Chromogenic agar plate (Brilliance TM UTI agar – Oxoïd, France), aerobic incubation
- Sabouraud agar plate (Becton Dickinson, Germany), aerobic incubation at 29 °C
- Brain heart infusion (bioMérieux, France), aerobic incubation

According to European Pharmacopoeia monograph of non sterile products, liquid oral formulations meet microbial requirements if the total aerobic microbial count are less than 10^2 cfu/ml, the total combined yeast/mould count are less than 10^1 cfu/ml and if there is no *Escherichia coli*.

3. Results and discussion

3.1. Methotrexate solution formulation

To insure a better homogeneity so as to secure administration, the choice was made to formulate a solution rather than a suspension. Thus injectable MTX Mylan® was used as it is available as a solution and does not contain any unsuitable ingredients for oral use (ingredients composition: injectable water, sodium chloride and sodium hydroxide). On the contrary, pure active MTX is a powder slightly soluble in water that forms suspension. Furthermore, we preferred to use an already made commercial injectable solution of pharmaceutical quality instead of a powder to be dissolved in order to limit the chemical contamination of

Table 1
MTX solution composition.

Component	Quantity
Methotrexate for injection 500 mg/20 ml	2.4 ml
Sodium bicarbonate	0.6 g
Ora Sweet®	7.5 ml
Sterile water	QS 30 ml