

Stability Of Levamisole Oral Solutions Prepared From Tablets And Powder

Fouad Chiadmi¹, Abdel Iyer¹, Salvatore Cisternino¹, Audrey Toledano¹, Joël Schlatter¹, Robert Ratiney², Jean-Eudes Fontan¹

¹Department of Pharmacy and Toxicology, University Hospital of Jean Verdier, avenue du 14 juillet, 95140 Bondy, France.

²Department of Pharmacy, University Hospital of Rene Muret – Bigottini, Avenue du Docteur Schaeffner, 93270 Sevran, France.

Key words: Levamisole, Oral solutions, Stability.

Received April 8 2005, Revised May 24, 2005, Accepted June 4 2005, Published 12 August 2005

ABSTRACT. Purpose: To study the stability of levamisole oral solutions (25 mg/mL) prepared from powder and tablets stored at $4 \pm 3^\circ\text{C}$ and $23 \pm 2^\circ\text{C}$ in amber glass prescription bottles. **Methods:** Levamisole 25 mg/mL solutions were prepared from commercially available 50-mg tablets or from pure powder in sterile water. Levamisole concentrations were determined in duplicate by a stability-indicating HPLC method at 0, 1, 2, 3, 4, 7, 14, 30, 60 and 90 days. The initial and final pHs of solutions were measured. **Results:** The recovery of levamisole from tablets was $100 \pm 2.1\%$. No color or odour changes were observed during the study period. The oral solutions prepared from powder were stable at least 90 days stored at 4 and 23°C . The oral solutions prepared from tablets were stable at least 90 days at 4°C and 15 days when stored at 23°C . The initial pH of solutions prepared from powder and tablets were 5.30 and 4.55, respectively. Initial and final pH values were significantly different ($p < 0.001$) for the two solutions. **Conclusions:** Levamisole 25 mg/mL oral solutions can be prepared from tablets or powder with sterile water for irrigation and stored for 90 days under refrigeration, taking account of the lack of microbiological contamination.

Corresponding author: Joël Schlatter, Department of Pharmacy and Toxicology, University Hospital of Jean Verdier, Avenue du 14 Juillet, 93140 Bondy, France, Tel: 33 1 48 02 66 03, Fax: 33 1 48 02 66 23, Email: joel.schlatter@jvr.ap-hop-paris.fr

INTRODUCTION

Treatment of recurrent idiopathic nephrotic syndrome in children is often complicated by the toxicity of the therapeutic regimen with corticosteroids, alkylating substances or cyclosporine. An alternative is the treatment with levamisole, a potent anthelmintic compound with immuno-modulating properties (1-3). In children, levamisole is given orally as 2 - 5 mg/kg daily or every other day depending on the patient response (4-6). Levamisole is commercially available only in 50-mg tablets under the trade name Ergamisol (Janssen Pharmaceutica). Levamisole is labelled by the United States Food and Drug Administration (FDA) as adjuvant treatment with fluorouracil but not labelled for the treatment of nephrotic syndrome. No liquid dosage form of levamisole is available. An oral solution would be highly desirable for children who are unable to swallow tablets and allows the dose to be easily adjusted. Thus, an oral solution could be helpful for levamisole administration. Few data exist on the levamisole stability in liquid form. The purpose of this study was to determine the chemical stability of levamisole in solution prepared from tablets and powder and stored at two temperatures (4 and 23°C) in glass prescription bottles over 90 days period.

METHODS

Formulation of levamisole solutions

One hundred 50-mg levamisole tablets^a were crushed to a fine powder in a glass mortar. Twenty mL of sterile water for irrigation^b were added and levigated into a paste. After 30 min, 100 mL of sterile water for irrigation were added and allowed to stand for 30 min. Then, the milky suspension was filtered through a 7- μm filter of paper^c to exclude the insoluble excipients. Sterile water^b was added to the clear solution in a volumetric flask to obtain a theoretical levamisole concentration at 25 mg/mL. The recovery of levamisole ($n = 5$) obtained from compressed tablets was measured. Another solution of levamisole 25 mg/mL was prepared with levamisole pure powder^d. The 5-g powder was weighed and mixed with water^b in a 200 mL volumetric flask. Ten millilitres from each levamisole solutions were transferred in 16 amber glass prescription bottles^e.

Storage of solutions

Six bottles of each formulation were stored at $4 \pm 3^\circ\text{C}$ and six other bottles were stored at room

temperature ($23 \pm 2^\circ\text{C}$) protected from light. Four bottles were used to determine the stability study at $60 \pm 5^\circ\text{C}$.

Sampling

From each bottle, a sample (100 μL) was taken and mixed with 900 μL of water^b. Then, 100 μL of this dilution was mixed with 300 μL of internal standard (1.5 mg/mL) and 600 μL of water in order to obtain a theoretical levamisole concentration at 250 $\mu\text{g}/\text{mL}$. The diluted sample was assayed in duplicate by high-performance liquid chromatography (HPLC), immediately after preparation (day 0) and after 1, 3, 4, 7, 15, 30, 60 and 90 days. The apparent pH of each solution was measured using a digital pH meter^f at the beginning and the end of the study. The appearance and color of the samples were assessed by observing samples against black and white backgrounds, and any changes of odor were noted at each time. The stability-indicating method was proven to ensure that potential degradation products did not interfere with levamisole and internal standard peaks.

HPLC Analysis

The HPLC method was adapted from Vandamme *et al* (7). The assay instrumentation required an isocratic pump^g, a manual injector^h with a 20 μL loop, an ultraviolet light detectorⁱ set at 235 nm, a C18 column^j set at room temperature and a recording integrator^k. The mobile phase consisted of potassium hydrogen phosphate^l 0.05 M and acetonitrile^m (85:15 v/v) used at a constant flow rate of 1 mL/min. Internal standard solution at 1.5 mg/mL was prepared by diluting quinine^o with water^b. Calibration curves were performed with standard solution diluted in mobile phase to yield levamisole base concentrations of 50, 100, 200, 300, 400 and 500 $\mu\text{g}/\text{mL}$, and 0.5 mg/mL of internal standard. The standard curve ($n = 5$) was constructed by plotting the peak-height ratio of levamisole to quinine against levamisole concentration, and was used for calculating the drug concentration of the sample. The method was shown linear ($r > 0.999$) in the range of 50 to 500 $\mu\text{g}/\text{mL}$. Three levamisole base control concentrations at 50, 200 and 500 $\mu\text{g}/\text{mL}$ were analyzed to determine intra- and inter-day coefficients of variation (CV). Each sample was injected in duplicate. The repeatability was assessed with 6 determinations at 3 levels (50, 200, 500 $\mu\text{g}/\text{mL}$) the same day. The intraday CVs were 3.79%, 1.18% and 0.63%, respectively. The reproducibility was assessed with 2 determinations on 3 days, at 3

levels (50, 200, 500 $\mu\text{g}/\text{mL}$). The interday CVs were 3.62%, 3.56% and 3.69%, respectively. The limits of detection (signal/noise=3:1) and quantification of levamisole were 0.36 and 1 $\mu\text{g}/\text{mL}$, respectively. The method was shown to be stability-indicating by adjusting the pH of levamisole solutions at 1 with acid (1.0 M HCl^p) and 12 with base (1.0 M NaOH^q), by oxidation with hydrogen peroxide^r and by storing oral solutions at 60°C . The stability indicating assay proved degradation of levamisole and no interfering peak. The retention times for levamisole and internal standard were 8 and 15 min, respectively. The duration of chromatogram was approximately 20 min (Fig. 1).

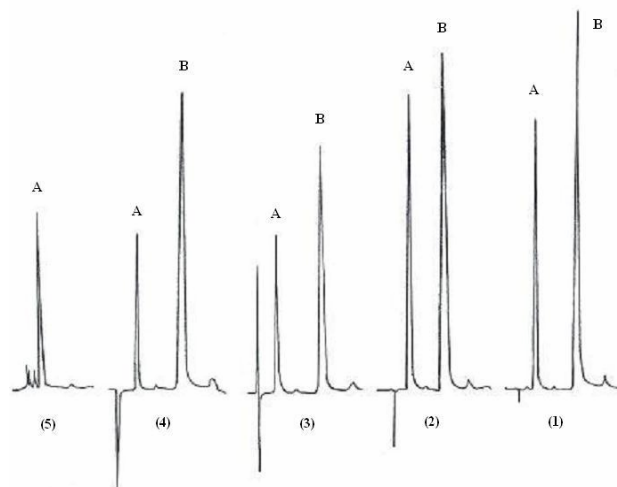


Figure 1: Chromatograms of a levamisole standard solution (1), an oral solution prepared from tablets stored at 25°C (2), solutions with acid (3) and base (4) and a solution with oxygen peroxide (5). A = levamisole; B = Quinine (internal standard).

Data analysis

The initial concentration of levamisole was defined as 100%, and sample concentrations were expressed as a percentage of the initial concentration remaining. The solution was defined as being stable if the drug concentrations were not $< 90\%$ of the initial drug concentration. The significance of any difference between initial and final pH values was evaluated by a Student's *t*-test ($\alpha = 0.05$).

RESULTS

The recovery of levamisole from tablets was $100 \pm 2.1\%$. In the oral solution prepared from powder, the mean concentrations of levamisole were $> 99\%$ of the initial concentration at 4°C and $> 96\%$ of the initial concentration at 25°C for the 90-days study period (Table 1). The mean concentrations of levamisole

were >94% of the initial concentration at 4°C and <64% of the initial concentration at 25°C over the study period for solution prepared from tablets (table 1). No change of appearance, color or odor was noted with any of the solutions. The apparent initial pH was significantly different between solutions prepared from tablets and those prepared from the powder (Table 1). The final pH values of solutions

prepared from tablets stored at 4°C and 25°C increased significantly ($p < 0.001$) by 1.07 and 1.57 units, respectively, as compared with the initial pH values. The final pH values of solutions prepared from powder stored at 4°C and 25°C increased significantly ($p < 0.001$) by 1.48 and 1.68 units, respectively, as compared with the initial pH values.

Table 1. Stability of levamisole solutions prepared from tablets and powder at 4 and 25°C.

Day	% Initial Concentration Remaining ^a			
	4°C		25°C	
	Tablets ^b	Powder ^c	Tablets ^d	Powder ^e
0	100 ± 1.85	100 ± 1.64	100 ± 0.53	100 ± 2.60
1	99.82 ± 3.19	99.98 ± 0.63	99.81 ± 1.36	99.86 ± 2.66
2	99.51 ± 3.00	99.95 ± 1.73	99.51 ± 0.96	99.80 ± 3.61
3	99.80 ± 0.96	99.94 ± 2.31	98.85 ± 2.28	99.78 ± 2.44
4	99.75 ± 2.56	99.96 ± 2.35	98.95 ± 1.27	99.92 ± 3.36
7	99.55 ± 2.85	99.88 ± 1.36	97.33 ± 2.21	99.79 ± 2.28
15	99.63 ± 0.79	99.86 ± 2.28	94.26 ± 1.59	99.54 ± 1.58
30	98.11 ± 1.52	99.81 ± 1.37	88.57 ± 2.33	99.10 ± 3.21
60	95.62 ± 3.50	99.26 ± 1.85	77.82 ± 1.96	98.11 ± 2.18
90	94.41 ± 3.22	99.23 ± 2.81	63.56 ± 3.32	96.77 ± 1.75

^aMean ± SD of 12 samples

^bAdded mean concentration was 25.52 ± 1.23 mg/mL, initial pH was 5.30 ± 0.03

^cAdded mean concentration was 24.89 ± 1.03 mg/mL, initial pH was 4.57 ± 0.03

^dAdded mean concentration was 25.35 ± 0.96 mg/mL, initial pH was 5.28 ± 0.02

^eAdded mean concentration was 25.22 ± 1.22 mg/mL, initial pH was 4.55 ± 0.01

DISCUSSION

The complete recovery of levamisole hydrochloride from tablets was related to its high water solubility reported to be 210 g/L (8). Levamisole solutions prepared from tablets and stored at 23°C appeared to be less stable than those prepared from powder. Refrigeration storage was shown to permit a better stability of levamisole solutions. Levamisole 25 mg/mL oral solutions prepared from powder and tablets stored at 4°C were shown stable at least 90 days and could be then used in clinical practice. This period of storage was conditioned by a possible microbiological contamination. With lack of preservatives in solutions, a period of 30 days stability seems more reasonable. Like temperature of storage, pH had an impact on the chemical stability

of levamisole. It was shown that the rate of decomposition of levamisole rapidly increased between pH 5 and 7 and at pH 8 it was about seventy times faster than at pH 2 (9-11). Excipients and pH of solutions prepared from tablets could probably explain the difference in stability. Thus, the use of the solution prepared from powder would be preferable in practice. The taste of solutions was bitter and would be masked by an additive.

CONCLUSION

An oral liquid solution of levamisole at 25 mg/mL prepared from tablets or pure powder in sterile water was shown chemically stable 90 days under refrigeration. These oral solutions appear to be widely used as an alternative to the administration of dry levamisole forms for the pediatric patients.

REFERENCES

- (1) Arvind Bagga, Amita Sharma, R. N. Srivastava. Levamisole therapy in corticosteroid-dependent nephrotic syndrome. *Pediatr Nephrol*, 11:415-417, 1997.
- (2) Tenbrock K, Muller-Berghaus J, Fuchshuber A, Michalk D, Querfeld U. Levamisole treatment in steroid-sensitive and steroid-resistant nephrotic syndrome. *Pediatr Nephrol*, 12:459-462, 1998.
- (3) Donia AF, Amer GM, Ahmed HA, Gazareen SH, Moustafa FE, Shoeib AA, Ismail AM, Khamis S, Sobh MA. Levamisole: adjunctive therapy in steroid dependent minimal change nephrotic children *Pediatr Nephrol*, 17:355-358, 2002.
- (4) MJ Kemper, O Amon, K Timmermann, H Altrogge, DE Muller-Wiefel. The treatment with levamisole of frequently recurring steroid-sensitive idiopathic nephrotic syndrome in children. *Dtsch Med Wochenschr*, 123:239-243, 1998.
- (5) L. Fu, CS Chi. Levamisole in steroid-sensitive nephrotic syndrome children with steroid-dependency and/or frequent relapses, *Acta Paediatr Taiwan*, 41:80-84, 2000.
- (6) U. Dayal, A.K. Dayal, J.C. Shastry, P. Raghupathy, Use of levamisole in maintaining remission in steroid-sensitive nephrotic syndrome in children, *Nephron*, 66:408-412, 1994.
- (7) TF Vandamme, M Demoustier, B Rollmann. Quantitation of levamisole in plasma using high performance liquid chromatography. *Eur J Drug Metab Pharmacokinet*, 20:145-149, 1995.
- (8) Levamisole Hydrochloride. The Merck Index on CD-ROM version 12.3. Merck and Co Inc, Whitehouse Station, NJ, USA, 2000.
- (9) NA Dickinson, HE Hudson, PJ Taylor. Levamisole: its stability in aqueous solutions at elevated temperatures. I. Isolation and identification of decomposition products formed in aqueous solutions of levamisole stored under nitrogen and oxygen at 100 degrees C. *Analyst*, 96:235-243, 1971.
- (10) NA Dickinson, HE Hudson, PJ Taylor. Levamisole: its stability in aqueous solutions at elevated temperatures. II. An assay specific for levamisole and applicable to stability studies. *Analyst*, 96:244-247, 1971.
- (11) NA Dickinson, HE Hudson, PJ Taylor. Levamisole: its stability in aqueous solutions at elevated temperatures. 3. A chromatographic and polarimetric study of the kinetics of degradation, *Analyst*, 96:248-253, 1971.

FOOTNOTES

^aLevamisole 50-mg tablets, ERGAMISOL®, Janssen Pharmaceutica, Beerse, Belgium, lot 02FB831. Tablets contained 59 mg of levamisole hydrochloride (= 50 mg levamisole base) cellulose, lactose, oil vegetable and silica.

^bSterile water for irrigation, VERSOL®, Aguetant, Lyon, France, lot F3133.

^cFilter paper N7, Cooperation Pharmaceutique Française (COOPER), Melun, France.

^dLevamisole powder, Synopharm Laboratory, Barsbüttel, Germany, lot 0307A108.

^eGlass prescription bottles reference 2506230, 125 mL, Cooperation Pharmaceutique Française (COOPER), Melun France.

^fModel pH302, Hanna Instruments, Tanneries, France.

^gModel LC-6A, Shimadzu Europe, Duisburg, Germany.

^hModel 7125, Rheodyne Europe GmbH, Bensheim, Germany.

ⁱModel SPD-6A, Shimadzu Europe, Duisburg, Germany.

^jNucleosil® (4.6-mm, 25 cm), Agilent Technologies, Massy, France.

^kModel CR-6A, Shimadzu Europe, Duisburg, Germany.

^lPotassium hydrogen phosphate, Merck KGaA, Darmstadt, Germany.

^mAnalytical grade acetonitrile, CHROMASOLV®, Sigma-Aldrich Chimie, Lyon, France.

ⁿEthanol 90% v/v, COOPER, Melun, France.

^oQuinine hydrochloride solution 30 mg/mL, AGEPS, Paris, France, lot T02081.

^pHydrochloride acid 37%, NORMAPUR®, VWR International SAS, Prolabo, Fontenay-sous-bois, France.

^qSodium hydroxide 1.0M, NORMADOSE®, VWR International SAS, Prolabo, Fontenay-sous-bois, France.

^rHydrogen peroxide 10%, COOPER, Melun, France.