Acta Pharmaceutica Sciencia
50: 241-245

Evaluation of Veratric acid Derivatives as Preservative in Aluminium Hydroxide Gel – USP

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Abstract

The veratric acid (3,4-dimethoxy benzoic acid) derivatives from our earlier study were subjected to preservative efficacy testing in an official antacid preparation, (Aluminium Hydroxide Gel – USP) against *Staphylococcus aureus MTCC 2901, Bacillus subtilis MTCC 2063, Escherichia coli MTCC1652, Candida albicans MTCC 227* and *Aspergillus niger MTCC 8189* as representative challenging microorganisms as per USP guidelines. The veratric acid derivatives, 8-quinolinyl veratrate (**P-2**) and phenyl veratrate (**P-3**) were found to be effective in preventing contamination of the product during the test period. This study showed the potential of veratric acid derivatives to be chosen as preservatives in pharmaceutical products.

Key words: Veratric acid, 8-quinolinyl veratrate, phenyl veratrate, preservative, Log CFU/mL

Introduction

Pharmaceutical products having high degree of water faces the problem of microbial spoilage which affects consumer safety (Zani *et al.* 1997). An antimicrobial preservative is added in a formulation during the manufacturing process and storage in order to prolong the shelf-life as well as for avoiding alteration and degradation of pharmaceutical products by contaminating pathogenic microorganisms (Denyer *et al.*1988). An ideal preservative should be effective at low concentration against all possible microorganisms, nontoxic and compatible with other constituents used in the preparation (Wilson *et al.*1998).

Preservative efficacy test (challenge test) includes artificial contamination of a formulation with a predetermined number of micro-organisms followed by periodic removal of samples at fixed time intervals which, after recovery in suitable media, are used for the viable count of the microorganisms present in the formulation. The standards regarding preservative efficacy, mainly proposed by Pharmacopoeias, necessitates harmonization between the different scientific units in the industry, and between the authorities responsible for evaluation and selection of suitable preservatives (Manou *et al.*1998).

The contribution of simple benzoic acid derivatives (methyl paraben, propyl paraben) as antimicrobial preservative created interest among us to search some new preservative

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compounds based on benzoic acid moiety. In the present paper we hereby report the preservative efficacy of most active antimicrobial 3,4-dimethoxy benzoic acid derivatives (Narasimhan *et al.* 2009) as a part of our ongoing medicinal chemistry research program.

Materials and Methods

Materials

Nutrient agar, nutrient broth, sabouraud dextrose agar and sabouraud dextrose broth were obtained from Himedia, Mumbai. Mannitol, methyl and propyl paraben were obtained from CDH, Mumbai.

Methods

Aluminium Hydroxide Gel USP 2004 was used as the pharmaceutical product for evaluation of preservative efficacy testing.

Preparation of Aluminum Hydroxide Gel-USP (Lachman *et al.* 1987)

Formula: Aluminium hydroxide gel -36 g; Mannitol -7 g; Methyl paraben - 0.2 g; Propyl paraben - 0.02 g; Saccharin - 0.05 g; Peppermint oil - 0.005 mL; Alcohol - 1 mL; Purified water q.s. - 100 mL.
The weighed quantity of aluminum hydroxide gel and mannitol were triturated with 50 mL of water in a mortar. Methyl paraben, propyl paraben, saccharin and peppermint oil were dissolved in alcohol and added to above mixture and triturated well. The volume was made up to 100 mL with purified water.
For preservative efficacy testing, the aluminium hydroxide gel was prepared using the preservatives mentioned in Table 1 by replacing methyl paraben and propyl paraben (Standard preservatives) from the formula mentioned above. The equimolar amount of selected preservatives (Table 1) were calculated with reference to the amount of methyl paraben (0.0013 mol) and added into the pharmaceutical products.

Table 1. Amount of selected preservatives added in the pharmaceutical products

Code	Preservative	Amount (g)
P-1	Veratric acid	0.237
P-2	8-Quinolinyl veratrate	0.402
P-3	Phenyl veratrate	0.335

Preservative efficacy testing in pharmaceutical products (USP 2004)
Aluminum hydroxide gel prepared with different preservatives was sterilized in autoclave at 120°C for 15 minutes. The products were then inoculated separately with 2 x 10^4 CFU/mL of *S. aureus, B. subtilis, E. coli, C. albicans* and *A. niger* and stored at room temperature (25°C). The CFU/mL of the product was determined at an interval of 0, 7, 14, 21 and 28 days. The experiment was performed in triplicate. The log values of number of colonies of microorganisms per mL (CFU/mL) along with their standard deviation (SD) (Table 2 – Table 6) were calculated and compared as in the light of USP 2004 guidelines.

Results and Discussions

According to USP, for antacid made with an aqueous base, preservative effectiveness are met if there is no increase from initial calculated count at 14^{th} and 28^{th} days in case of bacteria, yeast and moulds. No increase is defined as not more than 0.5 $\log_{10}$ unit higher than the previous value measured.

For B. subtilis: The results are presented in Table 2. The parent compound veratric acid (**P-1**) was effective against *B. subtilis* within the prescribed USP limits. The derivatives 8-quinolinyl

veratrate (**P-2**) and phenyl veratrate (**P-3**) were found to be effective on 14th day (0.000 ± 0.17, 0.000 ± 0.17) and 28th day (0.000 ± 0.00, 0.301 ± 0.00) as the log results were within the prescribed USP standards. The standard preservative was active on 14th day (0.000 ± 0.00) but fails to meet the required limit on 28th day (0.778 ± 0.03).

Table 2. Bacterial count (CFU/mL) of *B. subtilis* in Aluminium Hydroxide Gel USP supplemented with preservatives

Preservative / Day	Log CFU/mL ± SD				
	0	7	14	21	28
P-1	0.477 ± 0.08	0.301 ± 0.08	0.301 ± 0.08	0.000 ± 0.00	0.477 ± 0.09
P-2	0.477 ± 0.09	0.301 ± 0.08	0.000 ± 0.17	0.301 ± 0.08	0.000 ± 0.00
P-3	0.000 ± 0.00	0.000 ± 0.17	0.000 ± 0.17	0.000 ± 0.00	0.301 ± 0.00
Standard	0.602 ± 0.05	0.477 ± 0.08	0.000 ± 0.00	0.000 ± 0.17	0.778 ± 0.03
Control	0.698 ± 0.04	0.602 ± 0.00	1.113 ± 0.01	0.301 ± 0.08	0.845 ± 0.03

For S. aureus: As per the results shown in Table 3, veratric acid (**P-1**) was found to be active against *S. aureus* on 14th (0.000 ± 0.00) as well as 28th day (0.301 ± 0.08). The test compounds 8-Quinolinyl veratrate (**P-2**) and Phenyl veratrate (**P-3**) have shown complete inhibition of bacterium on 14th day (0.000 ± 0.00, 0.000 ± 0.17) as well as on 28th day (0.000 ± 0.00, 0.000 ± 0.00), so they passes the preservative effectiveness test. Standard showed complete inhibition on 14th day (0.000 ± 0.00) and less than 0.5 $\log_{10}$ unit increment of CFU/mL on 28th day (0.477 ± 0.09) from its previous values and hence meets the USP guidelines for preservative efficacy test against *S. aureus.*

Table 3. Bacterial count (CFU/mL) of *S. aureus* in Aluminium Hydroxide Gel USP supplemented with preservatives

Preservative / Day	Log CFU/mL ± SD				
	0	7	14	21	28
P-1	0.000 ± 0.00	0.000 ± 0.17	0.000 ± 0.00	0.000 ± 0.00	0.301 ± 0.08
P-2	0.000 ± 0.17	0.000 ± 0.00	0.000 ± 0.00	0.301 ± 0.08	0.000 ± 0.00
P-3	0.778 ± 0.03	0.301 ± 0.08	0.000 ± 0.17	0.301 ± 0.00	0.000 ± 0.00
Standard	0.602 ± 0.05	0.301 ± 0.08	0.000 ± 0.00	0.301 ± 0.00	0.477 ± 0.09
Control	0.903 ± 0.02	0.477 ± 0.08	0.602 ± 0.00	0.778 ± 0.03	0.845 ± 0.00

For E. coli: In case of *E. coli*, veratric acid (**P-1**) and both selected veratric acid derivatives (8-Quinolinyl veratrate (**P-2**) and Phenyl veratrate (**P-3**)) were found to be active as the logCFU/mL values were within the pharmacopoeial limits on 14th day (0.301 ± 0.08, 0.000 ± 0.17, 0.000 ± 0.00) as well as on 28th day (0.778 ± 0.03, 0.000 ± 0.00, 0.301 ± 0.00). The standard fails to meet the limits on 14th day (0.602 ± 0.05) but meets the limit on 28th day (0.698 ± 0.04). The results are presented in Table 4.

Table 4. Bacterial count (CFU/mL) of *E. coli* in Aluminium Hydroxide Gel-USP supplemented with preservatives

Preservative / Day	Log CFU/mL ± SD				
	0	7	14	21	28
P-1	0.477 ± 0.08	0.301 ± 0.00	0.301 ± 0.08	0.698 ± 0.04	0.778 ± 0.03
P-2	0.000 ± 0.00	0.000 ± 0.17	0.000 ± 0.17	0.301 ± 0.08	0.000 ± 0.00
P-3	0.000 ± 0.17	0.301 ± 0.08	0.000 ± 0.00	0.301 ± 0.08	0.301 ± 0.00
Standard	0.778 ± 0.03	0.000 ± 0.00	0.602 ± 0.05	0.301 ± 0.08	0.698 ± 0.04
Control	0.845 ± 0.03	0.602 ± 0.05	0.778 ± 0.03	0.954 ± 0.02	1.041 ± 0.02

For C. albicans: Veratric acid (**P-1**), 8-quinolinyl veratrate (**P-2**) and phenyl veratrate (**P-3**) were found to be effective on 14th day (0.301 ± 0.00, 0.000 ± 0.00, 0.301 ± 0.08) and 28th day (0.000 ± 0.17, 0.000 ± 0.17, 0.301 ± 0.00) as the log results were within the prescribed USP criteria. The standard also meets the USP limits and the test compounds showed results comparable to that of standard as shown in Table 5.

Table 5. Fungal count (CFU/mL) of *C. albicans* in Aluminium Hydroxide Gel-USP supplemented with preservatives

Preservative	Log CFU/mL ± SD				
	0	7	14	21	28
P-1	0.301 ± 0.08	0.301 ± 0.00	0.301 ± 0.00	0.301 ± 0.08	0.000 ± 0.17
P-2	0.000 ± 0.00	0.301 ± 0.08	0.000 ± 0.00	0.000 ± 0.17	0.000 ± 0.17
P-3	0.000 ± 0.00	0.477 ± 0.08	0.301 ± 0.08	0.301 ± 0.08	0.301 ± 0.00
Standard	0.301 ± 0.08	0.698 ± 0.04	0.602 ± 0.05	0.778 ± 0.03	0.000 ± 0.00
Control	0.477 ± 0.09	0.778 ± 0.03	0.845 ± 0.03	0.845 ± 0.03	0.903 ± 0.02

For A. niger: In case of Veratric acid (**P-1**), 8-quinolinyl veratrate (**P-2**) and phenyl veratrate (**P-3**) the increment in $\log_{10}$ CFU/mL at both 14th (0.301 ± 0.08, 0.000 ± 0.00, 0.301 ± 0.00) and 28th day (0.000 ± 0.00, 0.000 ± 0.17, 0.000 ± 0.17) was within the $0.5\log_{10}$ unit increment limit prescribed by USP 2004, so they passes preservative effectiveness test and have shown better activity as compared to the standard preservatives. The results are presented in Table 6.

Table 6. Fungal count (CFU/mL) of *A. niger* in Aluminium Hydroxide Gel- USP supplemented with preservatives

Preservative / Day	Log CFU/mL ± SD				
	0	7	14	21	28
P-1	0.477 ± 0.09	0.698 ± 0.04	0.301 ± 0.08	0.000 ± 0.17	0.000 ± 0.00
P-2	0.000 ± 0.00	0.301 ± 0.08	0.000 ± 0.00	0.301 ± 0.00	0.000 ± 0.17
P-3	0.301 ± 0.08	0.301 ± 0.08	0.301 ± 0.00	0.477 ± 0.08	0.000 ± 0.17
Standard	0.301 ± 0.08	0.301 ± 0.00	0.698 ± 0.04	0.000 ± 0.00	0.477 ± 0.08
Control	0.698 ± 0.04	1.079 ± 0.01	0.954 ± 0.02	1.000 ± 0.00	1.079 ± 0.01

The results of preservative efficacy test indicated that the test compounds, 8-quinolinyl veratrate (**P-2**) and phenyl veratrate (**P-3**) were found to be active against all the tested microbial strains under the standard test conditions prescribed by USP 2004.

Conclusion

The veratric acid derivatives selected for preservative efficacy testing have shown promising results. Both veratric acid derivatives viz. 8-quinolinyl veratrate (**P-2**) and phenyl veratrate (**P-3**) were found to be active against all the tested microbial strains under the standard test conditions as per USP 2004. The above criteria is supported by the log CFU/mL values of 8-quinolinyl veratrate (**P-2**) for 0 – 28th day viz. 0.000 – 0.000 (*S. aureus*), 0.477 – 0.000 (*B. subtilis*), 0.000 – 0.000 (*E. coli*), 0.000 – 0.000 (*C. albicans*), 0.000 – 0.000 (*A. niger*) and phenyl veratrate (**P-3**) for 0 – 28th day viz. 0.778 – 0.000 (*S. aureus*), 0.000 – 0.301 (*B. subtilis*), 0.000 – 0.301 (*E. coli*), 0.000 – 0.301 (*C. albicans*), 0.301 – 0.000 (*A. niger*) which were according to the prescribed USP criteria. The results of preservative efficacy testing indicated that both 8-quinolinyl veratrate (**P-2**) and phenyl veratrate (**P-3**) have the potential to be chosen as a pharmaceutical preservative.

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Received: 28.11.2008
Accepted: 29.12.2008