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A N I N T E R N A T I O N A L J O U R N A L

Sensory Evaluation of Albendazole Suspensions

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ABSTRACT

Sensory analysis was used in the albendazole suspension stability study. Three formulations were prepared and stored for 1, 3, and 6 months at 4 ± 1, 26 ± 1, 37, 50 and 65 ºC. Samples were evaluated through the difference from control sensory test using 24 trained judges in individual cabins. Although albendazole content was not altered in the conditions studied, sensory test showed differences between control and stored samples, except for one of the formulations stored under refrigeration for one month. These results have shown that the sensory evaluation is an important tool for quality control of pharmaceutical preparations, in association with chemical, physicochemical and microbiological tests.

Key words: Albendazole, sensory analysis, high performance liquid chromatography

INTRODUCTION

Albendazole is a potent antihelmintic benzimidazole, widely used in the human helmintiasis treatment due to its efficiency against all helmint classes which usually infest animal (Cook, 1990; Gasparine, 1995; Liu, 1986). It is also the drug of choice for neurocysticercosis treatment (Bandres et al., 1992; Cook, 1990; Gasparine, 1995; Liu, 1986). Commercially, it is available in tablets and suspension (DEF, 1999/2000). The choice for suspension instead of tablets or capsules is frequently controlled by patient acceptance. Although solid pharmaceutical formulations are usually given to adults, children are more easily treated with an adequately flavored suspension.

Assurance of therapeutic potency and microbiological safety of pharmaceutical preparations are evaluated through chemical, physicochemical and microbiological tests. However, although a medication may be ideal from the pharmacothecnical point of view, it may be deficient in relation to patient acceptance. The success of any medication, particularly of a liquid oral preparation is an important factor, which the manufacturer should take into consideration during the product development because the therapeutic effect will only be achieved if the patient accepts the sensory attributes of the formulation and completes the treatment. The product appearance and flavor, regardless of its shelf life, must be fresh, agreeable and elegant since any changes in one of these aspects, e.g.

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color alterations, may cause the patient loose confidence in that product.

The methodology through which the sensory attributes are evaluated is the Sensory Analysis. The word "sensory" derives from Latin, meaning "senses". This type of analysis has the advantage that the judges who take part in the tests carry with themselves the necessary measuring instruments, that is the five senses, thus including in the analysis product appearance (color, size and shape), flavor, odor, consistence, texture, sound (Anzaldúa-Morales, 1994) as well as transport and administration facilitation. This technique can be used in food, cosmetics, drug, cleaning products, paint and other industry, having in mind the product development, detection of lot to lot variations, change of process, cost reduction, quality control and drug stability control (Anzaldúa-Morales, 1994; Reyes-Vega et al., 1995; Meilgaard et al., 1999).

At present, researchers in the pharmaceutical field are using sensory evaluation in drug stability study by discriminative tests, and for the selection of the best formulation and flavor, preference tests are being applied (Reyes-Vega et al., 1995; Reyes-Vega et al., 1996; Valdés-Silva et al., 1997).

The objective of the present work was to apply the sensory test of difference from control in the study of albendazole suspension stability as well as to show that the sensory analysis could be an useful methodology in quality control of oral liquid pharmaceutical preparations.

MATERIAL AND METHODS

Samples

Three albendazole suspension formulations were used in this research. The composition of formulations is shown in Table 1.

Table 1 - Composition of albendazole suspension formulations

Compounds	Formulation		
	1	2	3
Albendazole	4.00 g	4.00 g	4.00 g
Viscocel®	2.50 g	-	-
Veegel®	-	-	2.00 g
Sodium carboxymethylcellulose (CMC)	0.40 g	1.30 g	0.50 g
Sorbitol 70%	10.00 mL	-	10.00 mL
Glycerine	-	5.00 mL	-
Tween 80®	0.20 mL	0.20 mL	0.20 mL
Nipagin®	0.20 g	0.20 g	0.20 g
Nipazol®	0.02 g	0.02 g	0.02 g
Ethyl alcohol	*	*	*
Saccharin	0.10 g	0.10 g	0.10 g
Orange flavor	0.30 g	0.30 g	0.30 g
Yellow colouring 5%	3 drops	3 drops	3 drops
Distilled water to	100.00 mL	100.00 mL	100.00 mL

*Enough amount

Preparation of suspensions

Suspension agent: Suspension agent was dispersed in about 30% in distilled water by mixing at 800 x g for formulation containing CMC and Viscocel®. Veegel® was dispersed in water at 60 ºC and then cooled until it reaches room temperature.

Albendazole dispersion: Albendazole was dispersed in glycerin for formulation 2, and for formulations 1 and 3 in sorbitol using porcelain mortar and pestle until total homogenization. This along with preservative solution, orange flavor, sodium saccharin previously prepared, and Tween 80® was added to the suspension agent dispersion under constant agitation. Final volume was reached with destilled water and after adding three drops of colouring agent, the liquid was shaken for

5 min. The complete final suspension was homogenized in colloidal mill for 3 min.

Sample preparation

Samples were prepared and stored in 15 mL amber glass bottles type III USP, Vick lids nº 23 at 4 ± 1, 26± 1, 37, 50 and 65 ºC for 1, 3 and 6 months in a way that the final time of storage was the same for all samples. Control samples for each formulation were prepared on the same day as the analysis.

Chemical Analysis

Albendazole content of samples was determined by High Performance Liquid Chromatography (HPLC). The chromatographic parameters used were: mobile phase: methanol - phosphate buffer 0.05 M (70:30; v/v); flow rate: 1.0 mL/min; attenuation: 0.032 AUSF; detection UV-254 nm; temperature: room temperature; column: Nucleosil® C18 (5 µ) - 150 x 4.60 mm (Phenomenex®) (Fregonezi-Nery et al., 2001).

Standard solution preparation and standard curve: A stock solution containing 25 µg/mL standard albendazole in formic acid (1 M) in methanol was prepared each day of sample analysis. Albendazole at 0.1, 1.0, 5.0, 10.0 and 15.0 µg/mL concentrations was obtained from two dilution of the stock solution in mobile phase. The standard curve was built each day of analysis through linear regression peak height versus albendazole concentration and it was linear (R^2 = 0.9997) under work concentration interval.

Sample preparation: Sample (5.0 mL), equivalent to 200 mg of albendazole, was quantitatively transferred to a 200 mL volumetric flask containing 50.0 mL of formic acid solution (1 M) in methanol. This solution was then agitated for ten minutes, diluted to the volume, filtered and diluted again in mobile phase, resulting in a 10.0 µg/mL albendazole solution.

Experimental design and statistical analysis: The design used was completely randomized, using the parameters time and storage temperature as treatment. Result significance was tested using analysis of variance (ANOVA) through Snedecor F Statistics and the Tukey's Test of means multiple comparison was applied using the SAS software (Statistical Analysis System, 1997).

Sensory Analysis

Twenty-four trained judges, in individual cabins, were used for the difference from control test application (Meilgaard et al., 1999).

Selection and training of judges: Judges were recruited after being questioned about their health conditions, availability and willingness to take part in the sensorial panel. Judges were trained not to take into consideration personal preferences, to understand the correct way of conducting the sensory analysis test and mainly to test the product being studied.

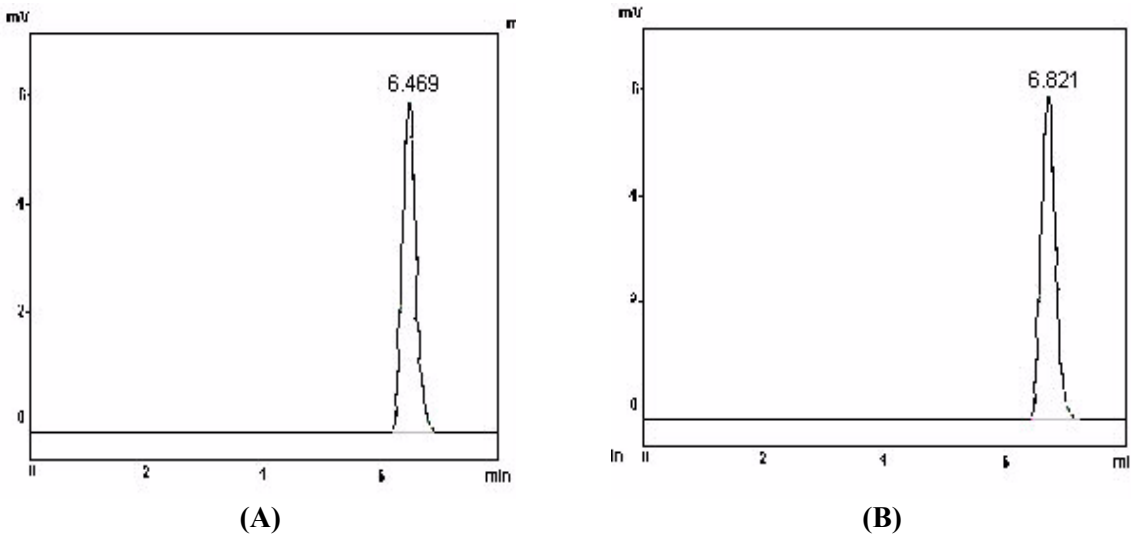
Test procedure: One sensory test for each formulation at each storage temperature was conducted, making 15 tests in 15 sessions to avoid judges fatigue. Judges were instructed to: evaluate samples in the sequence presented (left to right); wash their mouth with water before starting the test; assess samples in global terms (appearance, odor, flavor and consistence); let the samples completely impregnate their mouth and tongue hold it for the time necessary to detect the difference and then discard it; remove residual taste rinsing the mouth with water and eating a piece of apple before testing the following sample. At each session, each judge received simultaneously one control sample labeled "C" in the first order plus the samples stored in different time and one blind control sample labeled with a three digits code in a random order. Judges were requested to assess each coded sample, comparing it to the control (C), and to point the degree of difference using a 10-point scale where 0 = no difference and 9 = extremely different.

Experimental design and statistical analysis: Sensory analysis was repeated twice in completely randomized blocks. Significance of the results was tested using analysis of variance (ANOVA) through Snedecor F Statistics. Sources of variations were: storage time, judges, interaction between storage time versus judges, and the residue. The Dunnett's Test was applied to compare each treatment mean to blind control treatment mean using the SAS software (Statistical Analysis System, 1997).

RESULTS AND DISCUSSION

The stability study through sensory evaluation for the three albendazole suspension formulations in the five given storage conditions was followed by measurements of albendazole content by HPLC.

Figure 1 shows the chromatograms of the albendazole standard solution at 10.0 µg/mL concentration (A) and of the sample (B) in the same concentration. Retention time of albendazole in standard solution was 6.4 min and in the samples, 6.8 min.



(A) **(B)**

Figure 1 - Chromatograms of the standard solution containing 10.0 µg/mL of albendazole (A), and of sample (B) in the same concentration. Conditions: mobile phase: methanol: phosphate buffer 0.05 M (70:30; v/v); column: Nucleosil® - C18 (5 µm) - 150 x 4.6 mm (Phenomenex®); detection UV – 254 nm; flow rate: 1.0 mL/min; attenuation: 0.032 AUFS.

Table 2 - Albendazole contents obtained by HPLC in formulations 1, 2, and 3 stored in different times and temperatures*

Storage Temperature (ºC)	Time (months)	Formulations 1	2	3
Control**		45.78[a]	40.55[a]	45.68[a]
4 ± 1	1	46.92[a]	40.62[a]	46.60[a]
	3	46.02[a]	41.80[a]	47.50[a]
	6	46.16[a]	41.10[a]	46.00[a]
26 ± 1	1	47.00[a]	40.90[a]	46.92[a]
	3	45.84[a]	41.64[a]	46.92[a]
	6	46.98[a]	42.06[a]	44.80[a]
37	1	46.16[a]	40.54[a]	46.16[a]
	3	47.12[a]	40.38[a]	47.24[a]
	6	46.74[a]	42.26[a]	46.66[a]
50	1	47.26[a]	40.88[a]	46.54[a]
	3	46.70[a]	40.70[a]	45.58[a]
	6	46.02[a]	39.72[a]	46.86[a]
65	1	47.22[a]	40.50[a]	44.64[a]
	3	47.30[a]	42.08[a]	45.82[a]
	6	46.30[a]	39.94[a]	45.40[a]

* Mean values of 2 repetitions with 2 determinations each, expressed in mg of albendazole/mL of suspension.
** Control = room temperature / zero days in storage. Mean on the vertical marked with the same letters do not differ significantly (Tukey's Test, p = 0.05).

Table 3 - Mean values for sensory analysis of formulation 1 stored in different times and temperatures.

Temperature (ºC)	Time (months)			
	Control[a]	1	3	6
4 ± 1[b]	1.04	3.27*	3.67*	4.56*
26 ± 1[b]	1.65	3.13*	3.29*	5.02*
37[b]	1.19	3.10*	3.46*	3.67*
50[b]	1.17	3.52*	4.29*	.60*
65[c]	0.84	2.29*	4.02*	6.43*

[a] Control = room temperature / zero days of storage.
[b] Mean obtained through 2 repetitions by 24 judges each
[c] Mean obtained through 2 repetitions by 22 judges each.
Mean on the horizontal marked with * differ significantly from control (Dunnett's Test, p = 0.01%).

Table 4 - Mean values for sensory analysis of formulation 2 stored in different times and temperatures.

Temperature (ºC)	Time (months)			
	Control[a]	1	3	6
4 ± 1[b]	0.90	1.69	4.23*	3.96*
26 ± 1[b]	0.88	2.50*	4.17*	5.08*
37[b]	0.69	3.21*	3.27*	5.13*
50[b]	0.75	3.63*	4.08*	6.73*
65[b]	1.00	3.19*	3.92*	7.85*

[a] Control = room temperature / zero days of storage.
[b] Mean obtained through 2 repetitions by 24 judges each
Mean on the horizontal marked with * differ significantly from control (Dunnett's Test, p = 0.01%).

Table 5 - Mean values for sensory analysis of formulation 3 stored in different times and temperatures.

Temperature (º C)	Time (months)			
	Control[a]	1	3	6
4 ± 1[b]	0.79	2.77*	3.88*	4.38*
26 ± 1[b]	0.73	3.10*	3.60*	4.08*
37[c]	0.73	2.50*	3.68*	4.59*
50[b]	0.81	3.88*	4.20*	4.79*

[a] Control = room temperature / zero days of storage.
[b] Mean obtained through 2 repetitions by 24 judges each
[c] Mean obtained through 2 repetitions by 22 judges each.
Mean on the horizontal marked with * differ significantly from control (Dunnett's Test, p = 0.01%).

Table 2 shows the results obtained in the analysis by HPLC of albendazole in formulations 1, 2, and 3 stored for different times and temperatures.

There was no significant difference between the albendazole contents due to increase of temperature and the exposition time at each temperature for all the three formulations. This indicated that there was no chemical alteration for albendazole in the stored suspensions. Tables 3, 4, and 5 show the results obtained in the sensory evaluation for formulations 1, 2, and 3 stored in the different conditions, respectively. Only formulation 2 stored in refrigeration for one month did not differ from the control sample. Sensory attributes of the suspensions were deteriorated during the storage time, and the difference between each sample and the control sample increased according to storage time and temperature.

In the sensory evaluations for formulation 1 stored at 65º C and for formulation 3 at 37º C, only the answers of 22 judges were used. Analysis of

variance of the results obtained with 24 judges showed significant F values for the interaction storage time versus judges. Thus, according to Stone and Sidel (1993) it was possible to point out which judges were causing the interaction.
Results obtained from these judges were taken out from the total, and the analysis of variance was done again. For formulation 3 stored at 65º C, the F value for interaction was also significant but it was impossible to identify which judges caused the interaction and the results could not be interpreted.
Comments from judges included reference to one particular perception related to bitterness and darkening of samples as storage time and temperature increased. It was also said that there was a reduction in viscosity for samples stored at 26 ± 1, 37, 50 and 65 ºC, and an increase in viscosity for samples stored at 4 ± 1 ºC.
Thus, although suspensions have not had their albendazole content altered in the conditions studied, sensory alterations were present. These results indicated that sensory evaluation, as well as chemical, physicochemical and microbiological tests would be necessary for quality control of drugs, mainly in the liquid oral pharmaceutical formulations. Sensory aspects of appearance, odor, flavor or consistence could influence the therapeutic effect since patients might not take a medication if the sensory attributes were of not their liking. Besides, deterioration in any sensory characteristic may lead to an interruption of the treatment.

CONCLUSION

In all storage conditions studied, albendazole in oral suspension was chemically stable, what did not happen to the sensory attributes of the formulations. This fact showed that the difference from control test could be a useful tool to determine shelf life for this type of pharmaceutical formulations.

RESUMO

Três formulações contendo albendazol foram preparadas e armazenadas por 6 meses nas temperaturas de 4± 1, 26±1, 37, 50 e 65 °C. Para avaliação sensorial aplicou-se o teste de diferença do controle, com a participação de 24 provadores treinados. Determinou-se o teor de albendazol por cromatografia líquida de alta eficiência utilizando como fase móvel etanol - tampão fosfato 0,05 M (70:30; v/v). Os resultados encontrados nesta pesquisa indicaram que a avaliação sensorial é importante no controle de qualidade de medicamentos além dos testes químicos, físico-químicos e microbiológicos. Este fato é evidente principalmente para preparações farmacêuticas de administração oral, uma vez que os aspectos sensoriais dos medicamentos podem influenciar o efeito terapêutico. O paciente pode não ingerir medicamentos que não apresentem atributos sensoriais agradáveis.

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