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INVESTIGATION OF STABILITIES OF SOME PARENTERAL PREPARATIONS DEPENDING ON THE CONDITIONS OF AUTOCLAVING

BAZI PARENTERAL PREPARATLARIN ISIYLA STERİLİZASYON KOŞULLARINA BAĞLI OLARAK STABİLİTELERİNİN İNCELENMESİ

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Temperature, process period and settlement order in the autoclave applied during the sterilization procedure of parenteral preparations, prepared under specific study conditions, by heat cause many stability problems. The samples prepared in our laboratory and commercial preparations containing three agents, namely aminophylline, furosemid and verapamil ,were used. They were subjected to sterilization process at 121°C for 20, 30 and 60 minutes. The changes that occurred due to different settlement order in the autoclave during sterilization has been established through organoleptic controls, pH change and the determination of the amount of the agent. Besides, the effect of the structure of autoclaves, differing in their capacities and sizes, the effect of the amount of agent was also controlled in this study. In our investigations, it was determined that formulations of aminophylline with a pH value of 8.8, of furosemid with a pH value of 9.2 and of verapamil with a pH value of 4.5 were more stable than the others. In addition it was found that furosemid was more stable at its peak pH value and aminophylline and verapamil at their base pH values. On the other hand, it was found that pursued by autoclaves that have different sizes and the different order of settlement in the autoclave, samples setting closer to the walls of the autoclave degraded more. The particular change in the degradation of the preparations was not obtained by using different types of autoclaves.

Keywords : *Stability; Injections; Autoclaving*

Özel çalışma koşullarında hazırlanan parenteral preparatların ısı ile yapılan sterilizasyon işlemleri sırasında uygulanan sıcaklık, işlem süresi ve otoklav içi yerleşim düzeni bir çok stabilite problemini de ortaya çıkarmaktadır. Aminofilin, furosemid ve verapamilden oluşan 3 etken maddenin ticari preparatları ve laboratuvarda hazırlanan örnekleri 121°C 20, 30 ve 60 dakika süre ile sterilizasyona tabi tutulmuştur. Sterilizasyon sırasında otoklav içi farklı yerleşime bağlı olarak ortaya çıkan değişiklikler, organoleptik kontroller, pH değişimi ve etken madde miktar tayini ile saptanmaya çalışılmıştır. Bu çalışmamızda ayrıca farklı kapasite ve boyutlardaki otoklavların yapısının etken madde miktarı üzerine etkisi de incelenmiştir. İncelemelerimizde aminofilinin pH 8.8, furosemidin pH 9.2 ve verapamilin pH 4.5 olan formülasyonlarının ısıyla sterilizasyon sırasında diğerlerinden daha stabil olduğu saptanmıştır. Ayrıca furosemidin kullanıldığı pH aralığının üst sınırında, aminofilin ve verapamilin ise alt sınırında daha stabil olduğu bulunmuştur. Diğer taraftan otoklav için yerleşim ve farklı büyüklükte otoklavların kullanımı ile gerçekleştirilen sterilizasyon işlemleri sonucunda otoklav duvarına yakın bulunan örneklerin daha fazla bozunduğu saptanmıştır. Ancak farklı yapıda otoklav kullanımının preparatların bozunması üzerinde belirgin değişikliğe neden olmadığı anlaşılmıştır.

Anahtar kelimeler : *Stabilite; Parenteral preparatlar; Otoklav sterilizasyonu.*

Introduction

Parenteral solutions can be sterilized by heat (steam), ethylene oxide and radition. During sterilization by heat, it is necessary to control the temperature, moisture and time (1).

The standard sterilization method of heat-resistant injectable solutions is autoclaving at 121°C for 15 minutes. These conditions are approximately equal to the combination of time and temperature that provide a reduction of 10^{12} ($D_{121} \geq 1$ min) in resistant spores, that is to say the spores with an ability to overkill (2).

The thermal stability of the products should insure their remaining safe and efficient not only after the sterilization but also through their shelf-lives. At high temperatures, some harmful reactions such as hydrolytic and oxidative can be gain speed in the solutions (3). Drug degradation that could give harm to the patients may occur in several ways. The primary way of degradation takes place through the reduction in the amount of the agent. Generally, the decrease should remain within the range of 10%. During sterilization

by heat, hazardous degradation products could appear as well. In such a case, the pharmacopoeia narrows the boundaries down to 5% and sets the limits of the tests performed depending upon the degradation products wherever this case might be see. Degradation products may be directly toxic or problematic. Even if this degradation is at a level low enough allowed by pharmacopoeia, it may create significant clinical effects (4).

For years, sterilization procedure has been carried out according to a certain protocol of sterilization. (For instance, 30 minutes at 115°C and 15 minutes at 121°C) (5). Yet, heating and cooling periods may prolong to insure exposure to that high temperature up to 24 hours, depending on the batch size, type and size of the packing materials (6). An optimum sterilization procedure should provide the most reliable sterility with minimum degradation. These conditions can be defined as the "operation range", stating the exposure period and temperature required for the sterilization of one batch of a parenteral preparation. Wang has pointed out that a non-isothermal method could be used to determine in advance the degree of degradation that could occur during autoclaving (7). In literature, there are many methods to increase the chemical stability of the product sterilized by heat. These are the selection of the appropriate buffer (8) and the formation of counter ions (9) as well as the non-isothermal method. Even in modern autoclaving procedures, periods of heating and cooling are taken as a basis and the samples found in the autoclave then are exposed to different temperatures for varying periods of time. Therefore, the investigators consider the last stage of the pocess as well as the initial value. However, time and temperature profiles required for each production are derived from the values obtained from thermocouples placed into the samples at certain sites of the autoclave (9).

Besides this, there are many studies demonstrating the autoclaving effect on the degradation of the agents (10-21).

The aim of this study was to investigate the effects autoclaving temperature, period and different settlements in the autoclave. The effect of using autoclaves that have different sizes on the samples prepared with peak and base pH values was also investigated. The controls were regarding the organoleptic changes, pH changes and changes in the amount of the agent.

Materials

Agents and excipients: Aminophylline (Sandoz), furosemid (Hoechst), verapamil (Bayer) and their commercial preparations, boric acid (Merck), sodium hydroxide (Merck), potassium dihydrogen phosphate (PDR), disodium hydrogen phosphate. 12 H_2O (Merck), sodium acetate (Merck), hydrochloric acid (BDH), glacial acetic acid (Riedel de Haen). All substances and solvents were of analytical grade.
Equipment: U.V. Spectrophotometer (Pye-Unicam, Sp8-100), pH meter (Beckman, H4), autoclaves (Mudel, Medexport, K100-2 and ETC, Pro-Genesis 9240) were used in this study.

Methods

The commercial preparations of aminophylline, furosemid and verapamil and their solutions were prepared at peak and base pH values that have optimal resistanse (Table 1). They were autoclaved at 121°C for periods of 20, 30 and 60 minutes using the horizontal autoclave (Fig. 1). Establishing the effect of different order of settlement in the autoclave was also aimed. On the preparations organoleptic, pH controls and investigations of changes in the amount of the agents after autoclaving were performed.

Before and after autoclaving, as an organoleptic controls, solutions have been visually observed on a surface with a counter tone to detect if there were any changes in color and clearness and presence of precipitation and glass particles.

The pH changes in solutions have been established by the measurement of pH values at different periods before and after autoclaving.

To determine the extent of degradation of the agents during different autoclaving periods, different settlement orders in the autoclave and autoclaves differing in structure and sizes, measurements have been made with the spectrophotometric analysis methods. These analysis have been performed for each agent as indicated in the British Pharmacopoeia 1988.

Results and Discussion

Investigation of organoleptic changes

It is well know that instability case attracting the utmost attention in physical and chemical stability examinations of parenteral preparations as well as in all other dosage forms stem from the physical appearance. The physical changes in the formulations prepared are demonstrated in Table 2.

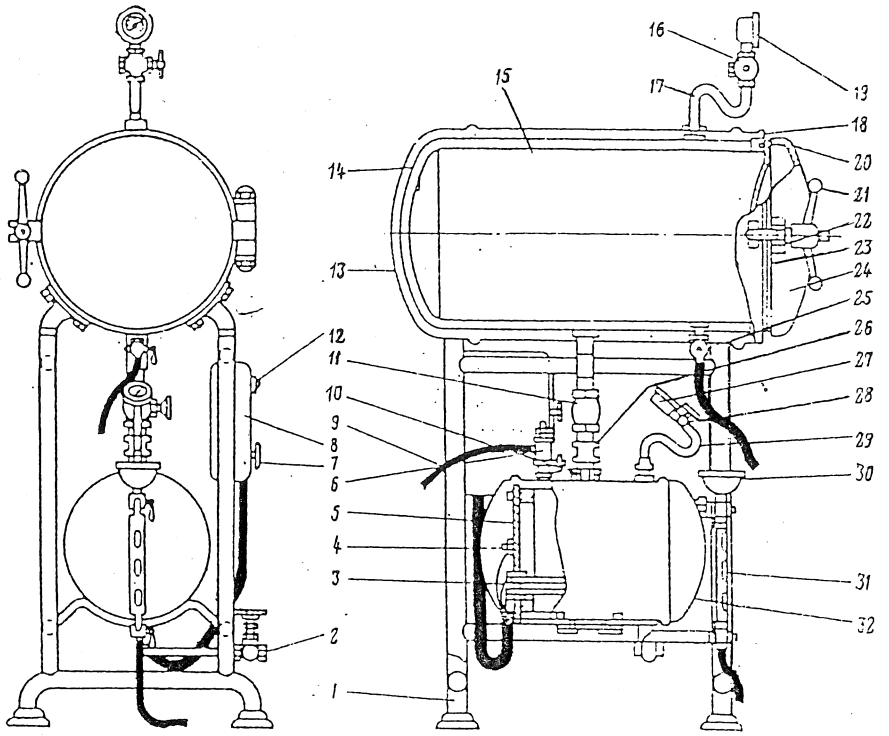
In aminophylline formulations, no change was observed, except for the formulation coded

Table 1. The formulations used in this study

Ingredients	Formulations												
	A.I (pH 8.8)	A.II (pH 10.0)	A.III (pH 8.0)	A.IV	A.V*	F.I (pH 8.0)	F.II (pH 9.2)	F.IV	F.V*	V.II (pH 4.5)	V.III (pH 6.0)	V.V	V.VI*
Aminophylline (g)	2.5	2.5	2.5	2.5	2.5%	-	-	-	-	-	-	-	-
Furosemide (g)	-	-	-	-	-	1.0	1.0	1.0	1.0%	-	-	-	-
Verapamil (g)	-	-	-	-	-	-	-	-	-	0.25	0.25	0.25	0.25%
0.2M (Boric acid + KCl) solution (ml)	25	25	-	-	-	25	25	-	-	-	-	-	-
0.2M NaOH (ml)	7.9	21.9	-	-	-	1.95	13.2	-	-	-	-	-	-
0.907% (w/v) KH_2PO_4 (ml)	-	-	34	-	-	-	-	-	-	-	88.9	-	-
2.39% (w/v) $Na_2HPO_4.12H_2O$ (ml)	-	-	66	-	-	-	-	-	-	-	10.1	-	-
KH_2PO_4 (g)	-	-	-	-	-	-	-	-	-	1.36	-	-	-
0.1M HCl (ml)	-	-	-	-	-	-	-	-	-	qs**	-	-	-
Purified water (qs)	100.0	100.0	-	100.0	-	100.0	100.0	100.0	-	100.0	-	100.0	-

* commercial preparations

** qs for pH 4.5



Horizontal Autoclave. Sectional View:
1- stand; 2- valve; 3- electric heater; 4- grounding bolt; 5- lid; 6- safety valve; 7- switch; 8- switch-panel; 9- hose; 10- nut; 11- valve; 12- pilot lamp; 13- shell; 14- steam chamber; 15- sterilizing chamber; 16- three-way cock; 17- siphon tube; 18- thrust ring; 19- pressure gauge; 20- rubber gasket; 21- clamp; 22- lever; 23- lid; 24- shell; 25- drain cock; 26- pipe branch; 27- pressure gauge; 28- three-way cock; 29- siphon tube; 30- funnel; 31- water-level indicator; 32- boiler

Fig.1. The horizontal autoclave used in this study

A. III depending on the autoclaving and sterilization period. In formulation A. III, it was seen that the turbidity called "Swirl" (22) has formed due to the sterilization period. It was suspected that this case was a result of the interaction between the materials such as glass and rubber stopper in the formulation.

As a result of prolonged autoclaving in furosemid formulations, evident turbidity and precipitation formation have been observed in F. III formulation. It is thought that this arises from the case called "Whiskers" (23) in the literature. The reason for this is that when kept at room temperature for a long time, the solution leaks from the small holes formed at the top of ampoule if it is not sealed properly. When this fluid evaporates, christals form. Sterilization at 121°C for 20 minutes is equal to storage at room temperature for 3 to 6 months (24).

In verapamil formulations no significant alteration was observed except for the formulation V.IV. In this formulation, it was observed that precipitation occurred by time. It is thought that this case arises from the reasons stated for A. III and F.III.

Investigation of pH change

It has been examined to what extent the pH values of the agents were affected depending on sterilization temperature and time. The agents had pH values close to the peak and base values of optimum pH range as pointed out in the literature (Fig. 2).

Table 2. The results of organoleptic controls in solutions depending on the sterilization period

Sterilization Period	Organoleptic Controls	Formulations												
		A.I	A.II	A.III	A.IV	A.V	F.I	F.II	F.IV	F.V	V.II	V.III	V.V	V.VI
Initial	Color	-	-	-	-	-	-	-	-	-	-	-	-	-
	Clearness	-	-	-	-	-	-	-	-	-	-	-	-	-
	Precipitation	-	-	-	-	-	-	-	-	-	-	-	-	-
	Glass particle	-	-	-	-	-	-	-	-	-	-	-	-	-
20 minutes	Color	-	-	-	-	-	-	-	-	-	-	-	-	-
	Clearness	-	-	- +	-	-	-	-	-	-	-	-	-	-
	Precipitation	-	-	- +	-	-	-	-	-	-	-	-	-	-
	Glass particle	-	-	-	-	-	-	-	-	-	-	-	-	-
30 minutes	Color	-	-	-	-	-	-	-	-	-	-	-	-	-
	Clearness	-	-	- +	-	-	-	-	-	-	-	-	-	-
	Precipitation	-	-	+	-	-	-	-	-	-	-	-	-	-
	Glass particle	-	-	-	-	-	-	-	-	-	- +	-	-	-
60 minutes	Color	-	-	-	-	-	-	-	-	-	-	-	-	-
	Clearness	-	-	+	-	-	- +	- +	-	-	-	-	-	-
	Precipitation	-	-	+	-	-	-	- +	-	-	-	-	-	-
	Glass particle	-	-	-	-	-	-	-	-	-	+	-	-	-

Changes:(-) none, (-+) very little, (+) evidently, (++) more excessive

After autoclaving for 20, 30 and 60 minutes it was observed that aminophylline formulations were not influenced much from sterilization for 20 minutes. However the longer the period was, the more changes occurred. For instance, whereas the pH values of formulations A.I and A. III decreased with an increase in the sterilization period, the pH values of other formulations increased (Fig. 2).

However in furosemid formulations, different autoclaving periods have not altered the pH much. At the end of autoclave sterilization for 60 minutes, a reduction of 0.11 (F.III) to 0.25 (F.I) has been observed in pH. As these values are in the range of measurement fineness, no evident degradation in pH could be pointed. Therefore it was concluded that the pH of the formulations was not affected much from the temperature and period of the sterilization (Fig. 2).

As for the verapamil formulations, it was seen that the pH values of the formulations except V.I and V.III, increased due to the period of sterilization (Fig. 2).

When the relation between the pH change and stabilities of the formulations was investigated, it was observed that the most stable formulations were A.I, F.II and V.II. On the other hand, it was found that formulations coded A.II, F.V and V.V were absolutely affected from the sterilization period and degraded. It is thought that these cases result from the fact that as pH increases, degradation increases too. Yet, although the pH value of formulation A.III was low, it degraded more than the formulation A.I. The reason of this could be that components forming the buffer in formulation A.I have increased the stability of the agent. In furosemid formulations, it was observed that as pH increases, stability increases

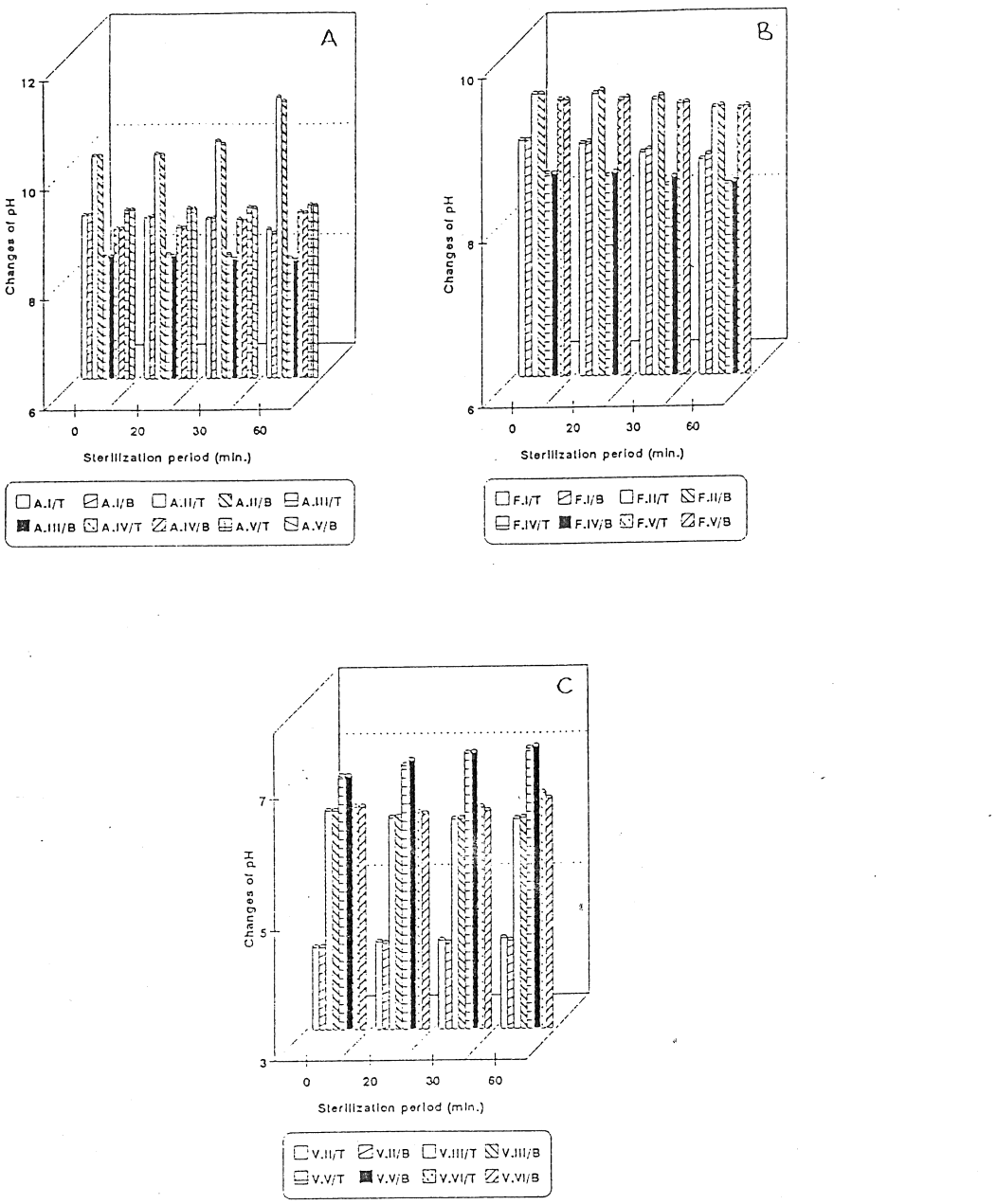


Fig. 2. The changes of pH in solutions that are (A) aminophylline, (B) furosemide and (C) verapamil depending on the sterilization period. (T= Above load matrix, B= Below load matrix)

too. However in this case while formulation F.V was expected to be the most stable formulation after formulation F.II, it was found to be the most degrading one. It is thought that as this was a commercial preparation and its formula structure was not know completely, this may be the result of the presence of an excipient in its structure.

The effect of having different positions in the autoclave on the stability of formulations has also been examined (Fig. 2). Consequently, it was seen that samples on the load matrix

Table 3. Amount of degradation of aminophylline solutions according to their settlement in autoclave as a result of the sterilization period. (The results are given as % remained. Mean ±s.d.)

Formulation	Serilization period(min.)	Settlement in Autoclave					
		*AF	AC	AN	BF	BC	BN
A.I	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	98.9 ± 2.3	99.6 ± 2.1	98.5 ± 3.7	99.3 ± 3.6	99.8 ± 4.4	99.0 ± 4.2
	30	98.7 ± 4.1	99.2 ± 2.7	98.4 ± 7.1	98.9 ± 5.3	99.4 ± 3.5	98.8 ± 3.9
	60	98.0 ± 4.0	98.5 ± 2.1	97.7 ± 6.8	98.3 ± 5.4	98.7 ± 2.3	98.0 ± 4.3
A.II	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	80.7 ± 2.1	91.2 ± 2.0	81.0 ± 3.0	99.6 ± 2.2	93.1 ± 2.0	91.2 ± 2.1
	30	79.9 ± 2.7	90.5 ± 3.9	79.6 ± 3.5	97.8 ± 2.1	92.0 ± 2.1	83.6 ± 3.0
	60	75.9 ± 4.2	87.2 ± 6.0	74.1 ± 5.2	97.0 ± 2.7	90.1 ± 2.9	76.3 ± 2.8
A.III	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	93.8 ± 3.0	92.0 ± 2.9	93.4 ± 2.1	97.8 ± 2.7	97.7 ± 2.2	95.0 ± 2.7
	30	92.9 ± 2.4	89.4 ± 3.0	92.3 ± 2.9	93.8 ± 3.0	89.8 ± 1.5	92.3 ± 3.4
	60	90.6 ± 4.4	87.6 ± 3.4	88.3 ± 4.7	91.2 ± 6.5	87.2 ± 5.4	90.5 ± 1.5
A.IV	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	98.5 ± 2.2	98.9 ± 4.9	98.5 ± 4.3	98.5 ± 2.2	99.2 ± 5.3	98.5 ± 4.5
	30	97.6 ± 3.8	98.7 ± 5.0	97.0 ± 5.9	97.8 ±3.0	98.9 ± 4.1	97.6 ± 5.4
	60	97.0 ± 5.5	98.2 ± 4.5	96.2 ± 3.9	97.0 ± 3.5	98.7 ± 5.9	96.8 ± 3.0
A.V	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	98.0 ± 5.0	99.0 ± 3.2	97.7 ± 3.2	98.3 ± 3.7	99.1 ± 5.7	97.9 ± 3.7
	30	92.0 ± 5.1	93.1 ± 4.1	92.7 ± 5.2	92.3 ± 4.0	93.8 ± 3.1	92.7 ± 1.9
	60	80.7 ± 1.5	88.0 ± 3.0	83.2 ± 5.0	83.2 ± 5.3	89.9 ± 3.9	83.7 ± 3.9

* AF:The farthest situation at the above of load matrix, AC: The center situation at above load matrix. AN: The nearest situation at above load matrix, BF: The farthest situation at the below of load matrix, BC: The center situation at below load matrix. BN: The nearest situation at below load matrix codified concerning the autoclave's cover.

above were influenced more depending on the sterilization period. It is thought that the steam's penetrating effect on the samples on the load matrix below is less and their being exposed to less heat may have led to this situation. However the formulations coded A.VI, F.I, and V.V. have degraded more, when they were on the load matrix below. It could be that as the samples were placed at the edges of the load matrix below during settlement into the autoclave, they were exposed to more theat and this may have caused the degradation, since the samples at the edges are exposed to more heat than those in the middle (9).

When the effect of sterilization temperature and period on the pH values of the preparations was investigated, it was concluded that there has been no direct and evident effect on the pH change. The change seen in the amount of agent depends on the pH value. It was not effected much from the order of settlement in the autoclave.

Table 4. Amount of degradation of furosemide solutions according to their settlement in autoclave as a result of the sterilization period. (The results are given as % remained. Mean ±s.d.)

Formulation	Sterilization period(min.)	Settlement in Autoclave					
		*AF	AC	AN	BF	BC	BN
F.I	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	98.6 ± 3.8	98.9 ± 5.1	98.5 ± 4.2	98.7 ± 3.7	99.0 ± 4.0	98.6 ± 2.3
	30	98.5 ± 4.0	98.7 ± 5.0	98.4 ± 5.9	98.7 ± 2.2	98.9 ± 5.0	98.3 ± 4.7
	60	97.6 ± 4.7	97.8 ± 3.9	97.6 ± 2.4	97.6 ± 2.2	98.1 ± 2.8	97.4 ± 3.6
F.II	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	99.9 ± 5.2	100.0 ± 4.6	99.9 ± 5.0	100.0 ± 5.7	100.0 ± 5.0	100.0 ± 5.5
	30	99.3 ± 2.3	99.8 ± 2.3	99.2 ± 4.6	99.7 ± 3.1	99.9 ± 3.4	99.5 ± 4.1
	60	98.7 ± 4.9	99.0 ± 5.2	98.5 ± 1.0	99.0 ± 5.5	99.2 ± 5.0	98.8 ± 4.0
F.IV	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	99.0 ± 6.6	99.3 ± 4.9	98.6 ± 7.2	99.2 ± 4.2	99.5 ± 5.3	98.9 ± 4.6
	30	99.0 ± 4.6	99.2 ± 6.9	98.6 ± 6.1	99.2 ± 7.0	99.5 ± 5.1	98.9 ± 4.7
	60	96.5 ± 5.0	96.8 ± 5.8	96.3 ± 7.1	96.9 ± 7.2	97.0 ± 4.3	96.7 ± 3.5
F.V	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	98.9 ± 5.2	99.2 ± 5.9	98.7 ± 5.7	99.0 ± 5.5	99.5 ± 5.6	99.0 ± 6.4
	30	97.5 ± 5.2	97.9 ± 5.7	97.2 ± 4.3	98.4 ± 4.3	98.6 ± 3.2	98.3 ± 5.0
	60	94.5 ± 5.9	95.0 ± 5.7	94.3 ± 4.3	95.2 ± 4.6	95.6 ± 4.7	94.9 ± 5.2

* AF: The farthest situation at the above of load matrix, AC: The center situation at above load matrix. AN: The nearest situation at above load matrix, BF: The farthest situation at the below of load matrix, BC: The center situation at below load matrix. BN: The nearest situation at below load matrix codified concerning the autoclave's cover.

Investigation of changes due to the sterilization period

In all aminophylline formulations approximately similar changes have occurred depending on the sterilization period. The most degrading formulation was the A.II coded alkali borate solution with a pH value of 10.0. The reason for this is thought to be the weakening of theophylline-ethylene diamine bonds in aminophylline because of the increase in pH and thus easier and more release of theophylline occurs. On the other hand the most resistant aminophylline formulation was the A.I coded with a pH value of 8.8. According to this, it was found that the formulations require to have a pH value closer to the base value of the stated pH range and they should be sterilized at 121°C for 20 minutes.

When the changes in furosemid formulations due to autoclaving period were examined, formulation F.II was found to be the most stable one. On the other hand the most degrading one has been F.V. In this case it was understood that contrary to the aminophylline formulations, furosemid formulations could be sterilized at 121°C for 20 minutes. Their pH values should approach to peak values of the stated pH range.

Verapamil formulations, too, were influenced from the temperature and period of sterilization. The least affected formulation was V.II coded phosphate buffer solution with a pH value

Table 5. Amount of degradation of verapamil solutions according to their settlement in autoclave as a result of the sterilization period. (The results are given as % remained. Mean ± s.d.)

Formulation	Sterilization period(min.)	Settlement in Autoclave					
		AF	AC	AN	BF	BC	BN
V.II	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	99.6 ± 2.4	99.6 ± 3.0	99.5 ± 2.8	99.9 ± 2.9	100.0 ± 4.3	99.7 ± 6.3
	30	99.0 ± 3.2	99.2 ± 2.3	98.3 ± 2.7	99.0 ± 3.0	99.2 ± 4.1	98.7 ± 4.0
	60	98.5 ± 3.4	98.7 ± 3.5	97.9 ± 3.0	98.5 ± 3.8	98.7 ± 3.2	98.3 ± 2.3
V.III	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	97.8 ± 2.1	98.1 ± 1.9	97.5 ± 1.7	99.1 ± 2.3	99.9 ± 2.0	98.5 ± 3.8
	30	96.6 ± 2.6	96.8 ± 3.6	96.3 ± 3.1	97.2 ± 2.4	97.4 ± 3.2	94.7 ± 2.0
	60	95.9 ± 3.0	96.2 ± 3.8	95.3 ± 3.2	96.2 ± 2.4	96.2 ± 3.2	96.1 ± 3.2
V.V	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	98.3 ± 2.2	99.1 ± 2.8	96.7 ± 2.7	98.8 ± 2.7	99.2 ± 3.5	96.9 ± 3.0
	30	97.6 ± 2.6	98.9 ± 2.0	95.4 ± 3.7	97.5 ± 3.9	99.2 ± 3.4	96.3 ± 4.1
	60	96.9 ± 1.9	97.9 ± 2.3	94.6 ± 2.1	97.2 ± 2.8	98.2 ± 2.7	95.5 ± 3.4
V.VI	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	94.4 ± 2.9	94.7 ± 2.3	94.2 ± 3.6	95.6 ± 3.7	95.8 ± 3.1	95.2 ± 4.1
	30	93.6 ± 2.4	94.0 ± 3.0	93.3 ± 2.1	94.2 ± 3.2	94.4 ± 2.6	94.0 ± 1.7
	60	92.4 ± 2.6	92.8 ± 2.0	91.9 ± 2.0	92.8 ± 3.4	93.1 ± 2.6	92.4 ± 2.8

* AF: The farthest situation at the above of load matrix, AC: The center situation at above load matrix. AN: The nearest situation at above load matrix, BF: The farthest situation at the below of load matrix, BC: The center situation at below load matrix. BN: The nearest situation at below load matrix codified concerning the autoclave's cover.

of 4.5. The most degrading one was the formulation V.VI. As this was a commercial preparation, this fact may arise from its being sterilized for the second time. The same case was valid for the formulation A.V that was the most degraded formulation of aminophylline during the second sterilization.

Consequently, all agents were affected from temperature and time during sterilization in the autoclave. The interaction increases in proportion to the sterilization period. The degree of degradation as a result of sterilization for 20 minutes was within the acceptable range. However prolonged high temperatures to which formulation was exposed during the cooling period of the autoclaving caused problems for all formulations.

The investigation of changes in the amount of agents depending on the settlement in the autoclave

It has been established how the positions of the formulations in the autoclave affected their stabilities. At the end of autoclaving of the samples placed on the above and below of load matrixes at 121°C for 20, 30 and 60 minutes, the results shown on Tables 3,4 and 5 were obtained.

According to these results, the samples at the center degrade less than those at the edges. There are also some differences at the edges of load matrix about their distance from the cover. The samples closer to the cover have degraded more. When the samples on the load matrixes above and below were compared,

Table 6. The results regard to the effect of sterilization period on degradation of drugs according to their different order settlement in the autoclave having a greater capacity. (The results are given as % remained. Mean ± s.d.)

Settlement in the autoclave	Sterilization period (min.)	Formulations												
		A.I	A.II	A.III	A.IV	A.V	F.I	F.II	F.IV	F.V	V.II	V.III	V.V	V.VI
Top load matrix	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	98.3 ± 4.2	81.7 ± 4.1	99.1 ± 4.1	98.7 ± 3.3	93.8 ± 2.2	97.9 ± 4.8	99.7 ± 3.3	97.3 ± 5.8	99.7 ± 5.7	99.4 ± 4.1	97.6 ± 3.5	97.0 ± 4.7	94.7 ± 5.0
	30	97.8 ± 4.2	79.5 ± 4.4	98.8 ± 5.3	92.7 ± 4.1	92.5 ± 5.2	96.6 ± 3.1	99.3 ± 2.2	96.4 ± 5.6	99.3 ± 4.6	98.5 ± 3.4	95.9 ± 5.8	96.0 ± 3.7	93.6 ± 4.8
	60	97.3 ± 3.7	75.7 ± 3.3	98.1 ± 4.8	88.3 ± 3.6	83.3 ± 3.5	94.8 ± 4.3	98..2 ± 3.2	94.0 ± 4.4	98.2 ± 4.3	96.4 ± 3.3	95.0 ± 5.1	93.9 ± 2.8	90.8 ± 5.9
Center load matrix	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	99.8 ± 4.3	91.2 ± 4.4	99.7 ± 4.1	98.9 ± 4.8	96.6 ± 4.5	98.1 ± 3.5	99.9 ± 5.7	97.6 ± 2.1	99.9 ± 4.7	99.6 ± 5.4	98.3 ± 1.9	98.2 ± 3.1	95.2 ± 2.9
	30	99.1 ± 3.2	90.0 ± 4.7	99.1 ± 4.5	94.1 ± 3.3	93.1 ± 5.5	97.4 ± 2.8	99.5 ± 3.9	97.6 ± 4.0	99.5 ± 5.5	98.9 ± 3.6	96.2 ± 3.0	96.4 ± 3.3	94.1 ± 3.4
	60	98.0 ± 4.5	87.0 ± 2.1	98.2 ± 3.6	91.2 ± 4.6	88.3 ± 2.1	95.4 ± 3.3	98.7 ± 3.2	93.3 ± 4.3	98.7 ± 3.7	97.0 ± 4.6	95.2 ± 3.2	94.5 ± 3.9	91.3 ± 4.8
Bottom load matrix	0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	20	98.7 ± 5.6	81.0 ± 4.1	98.7 ± 5.8	98.6 ± 3.4	91.9 ± 2.4	97.3 ± 4.1	99.5 ± 2.7	97.1 ± 3.3	99.5 ± 3.0	99.0 ± 4.2	97.2 ± 3.2	96.3 ± 5.4	93.9 ± 3.2
	30	98.5 ± 3.6	79.0 ± 5.3	98.5 ± 4.4	89.5 ± 3.5	91.7 ± 4.2	96.6 ± 1.9	99.0 ± 3.7	96.4 ± 4.1	99.0 ± 2.1	98.1 ± 3.5	95.3 ± 5.4	95.3 ± 4.4	93.0 ± 4.2
	60	97.7 ± 2.7	73.8 ± 4.1	97.7 ± 3.3	87.7 ± 4.9	80.0 ± 4.4	93.3 ± 5.0	98.0 ± 0.9	93.4 ± 5.3	98.0 ± 2.8	95.2 ± 3.8	94.6 ± 4.8	93.8 ± 3.4	90.2 ± 2.6

it was seen that those on the load matrix above degraded more. It can be reckoned that these differences might arise from the air left in the autoclave during sterilization. However, during the initial investigations of the study the process period of the autoclave has been determined to eliminate such cases and to ensure that all agents are exposed to the same temperature for the same time.

Furthermore, one thinks that as the loading matrix was completely full and the material sterilized was too small and they were placed into the autoclave not on perforated grids but on trays and therefore the hot steam may not have penetrated through the whole material completely. Thus, since the hot steam got closer to the samples at the edges of load matrix when compared to those at the center, the samples at the center have degraded less.

One may also think that the differences between the load matrixes result from the fact that as the samples on the load matrix below are closer to the steam feeding line, they are expected to degrade more. Yet the results have shown just the opposite development. The reason for this may be that, the samples have been placed on two load matrixes in the autoclave and the distance between the two trays has decreased and hot steam could not be intense enough to spread and could not make a full impact.

The investigation of stability changes depending on the use of different autoclaves

To investigate the effect of the use of different autoclaves on degradation, ETC, Pro-Genesis 9240 type autoclave was used. It had a greater capacity than the other horizontal autoclave, but its function was the same. The results obtained can be seen on Table 6.

According to the results obtained, in the autoclave having a greater capacity more degradation was observed than in the one used for pilot production. The volume of the autoclave was too large and the measured samples were flacons of 10 ml. They occupied a place much less than their loading capacities and thus they were exposed to more heat.

In addition, it was established that among the samples having different positions in the autoclave, those on the load matrix below degraded more. The reason for this may be that even though the samples placed on the trays with fine holes were supported in big bottles having a 500 ml capacity, they were exposed more to the hot steam coming from below.

Conclusion

According to the results obtained the formulations prepared with three active agents at the peak and base pH values and their commercial preparations were affected at different rates by the different sterilization periods. The deviation of the stability that occurred with the change at the sterilization period has also changed depending on such effects as whether the preparations are at the center or at the edges of load matrix of the autoclave and whether the autoclaving is being made in autolaves with different capacities.

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