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Research paper

Stability of Binary Mixtures of Drugs at Different Concentrations and Temperatures

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ABSTRACT

***Background and Objective**: In order to avoid separate injections, admixtures of drugs are frequently used in palliative care setting. The objective of this work is to study the stability of binary mixtures of drugs (ondansetron and midazolam) at different concentrations and temperatures all prepared in 0.9% NaCl and stored into infusers and glass in all cases protected from light.*

***Materials and Methods**: Two methods have been employed to determine periodically the concentration of each drug in the mixtures (HPLC-UV and UV-vis spectrophotometry methods). The concentrations of the samples were: 0.1 mg mL^{-1} – 0.1 mg mL^{-1} and 0.5 mg mL^{-1} – 1.0 mg mL^{-1} of ondansetron and midazolam respectively; temperature of storage 25ºC and 37ºC. NaCl 0.9% was used as diluent in all cases. The containers to store each mixture were three infusers and glass.*

***Results**: All solutions were initially clear and colourless but visible particles appear, in all cases, into the infusers after two days since their preparation.*

***Conclusions**: The admixture of ondansetron and midazolam in NaCl 0.9% is recommended to use for a maximum of one day, at the concentrations evaluated, over time it tends to precipitate. Infuser conditioning decreases stability with respect to other conditions materials, so other stability studies may not be extrapolated if stored under different conditions.*

***Keywords:** Ondansetron; Midazolam; Compatibility; Stability; Palliative Care*

Introduction

Palliative care is high quality health care and support for people living with a life-limiting illness and their families. Palliative care helps people to live as well as they can by managing pain and symptoms to ensure their quality of life is maintained as the illness progresses. Palliative care identifies and treats symptoms and issues associated with life-limiting illness which may be physical, emotional, spiritual or social. Palliative care is a family-centred model of care, meaning that family and carers can also receive practical and emotional support. Palliative care is about maintaining quality of life. The aim of palliative care is neither to hasten nor postpone death. Rather, the focus is on living as well as possible, for as long as possible [1].

Patients in the end of life may present with multiple symptoms, depending on the nature and stage of their disease. In the case of cancer patients, the location of the tumour, its grade, local extension and metastasis determine the symptoms. National studies on the prevalence of symptoms refer mainly to cancer patients. In these series, pain, asthenia and anorexia appear in more than 70% of the patients [2].

The use of drugs to control symptoms in PC has some special characteristics that must be considered. Patients with advanced or terminal illness constitute a particularly vulnerable population. Your environment and the different psychological factors can have a great influence on your physical well-being and on your response to drug treatment, a response that will sometimes be unpredictable. These patients are often elderly, frail or with multi-organ involvement and polymedicated, with the consequent risk of interactions and iatrogenesis.

The challenge for professionals and caregivers is to treat symptoms effectively, maintaining maximum patient comfort and minimizing adverse effects and inconveniences of treatment or very complex guidelines.

The choice of the route of administration depends on factors related to the patient, the drug and organizational factors (availability of formulations, human resources, etc.).

The main route of administration in PC is oral, since it is a simple, non-invasive and acceptable route for most patients. However, there are situations in which oral administration of drugs is not possible (for example, when the patient has nausea and vomiting, seizures, dysphagia or intestinal obstruction). In cases where oral drug administration is not possible, alternative routes of administration need to be used. Examples of these alternative routes are the intravenous route, the intramuscular route, the rectal route, the transdermal route, the sublingual route or the subcutaneous route [3].

At present, the subcutaneous route is considered the most appropriate of the alternative routes, especially at the community level in order to maintain the patient's autonomy and be able to live their last days in their natural environment.

The subcutaneous route is not very aggressive and allows self-administration by the patient or by the patient's family [4]. In many cases it is necessary to administer more than one drug, so mixing them in the same infuser would be the best alternative. However, there are few published data on the stability of mixtures, and even less if we focus on data on the physical-chemical stability of mixtures in infuser-type systems and preserved in conditions of temperature and light similar to those of healthcare practice [5-13].

Ondansetron, (RS) -9-methyl-3- [(2-methyl-1H-imidazol-1-yl)-methyl] -1,2,3,9-tetrahydro-4H-carbazol-4-one-mono hydrochloride, is a selective serotonin 5-HT3 receptor antagonist with antiemetic activity. It is used in the management of nausea and vomiting induced by cytotoxic chemotherapy and radiotherapy. The antiemetic activity of the drug is brought about through the inhibition of 5-HT3 receptors present both centrally (medullary chemoreceptor zone) and peripherally (GI tract). This inhibition of 5-HT3 receptors in turn inhibits the visceral afferent stimulation of the vomiting center, likely indirectly at the level of the area postrema, as well as through direct inhibition of serotonin activity within the area postrema and the chemoreceptor trigger zone [14-16].

Midazolam is a short-acting benzodiazepine and is used clinically for sedative purposes prior to minor medical procedures and surgery. Benzodiazepines are drugs with hypnotic, anticonvulsant and tranquilizing properties and are prescribed worldwide for the therapy of anxiety, sleep disorders, and convulsive attacks.

Midazolam is indicated for the acute management of aggressive or delirious patients and less for the acute management of seizures such as status epilepticus. Occasionally is used as a hypnotic, especially in hospitals. Hospital pharmacies are required to centrally prepare parenteral drugs and other pharmaceutical compounding, ensuring that the preparation they produce remain stable and thus retain all of their properties throughout storage time and right up to the point when they are administered to the patient. Pharmacies are also required to respond to therapeutic needs not covered by the pharmaceutical industry, as in the case of mixture off different drugs in the same recipient. In a previous work published by us there are a revision about the stability's studies of midazolam alone and in mixture with other drugs [17].

This paper carries out the study of the compatibility and stability of ondansetron-midazolam by two procedures (HPLC-UV and UV-vis spectrophotometry methods), at two levels of concentrations and stored at two different temperatures and containers all of these protected from the light.

Materials and Methods

Materials: Commercial ondansetron ampoules of 2 mg mL^{-1} (Fresenius Kabi, Spain). Midazolam ampoules of 5 mg mL^{-1} (Normon). Sodium chloride 0.9% was obtained from Fresenius Kabi, Spain. Methanol (HPLC grade) was purchased from Merck-Corporation (Darmstadt, Germany). Other chemical and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). High purity water (resistivity 18.2 MΩ cm) obtained by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout this work.

Preparation of sample solutions: The doses of ondansetron and midazolam assayed in the study were chosen taking into consideration those more frequently used by the units of palliative care in our region. The doses assayed were 0.1 mg mL^{-1}–0.1 mg mL^{-1} and 0.5 mg mL^{-1}–1.0 mg mL^{-1} of ondansetron and midazolam respectively, which were prepared in 0.9% normal saline for injection and stored at two temperatures, 25°C and 37ºC each one, employing a bacteriological and culture oven with temperature and time regulation and digital reading, Selecta (INCUDIGIT 19L 001246). Each of these four alternatives were prepared in triplicate in elastomeric infuser (type Baxter) and protected from light and also in glass.

Preparation of standard solutions: For the first mixture mentioned above (0.1 mg mL-1 ondansetron-0.1 mg mL-1 midazolam), five standards were prepared by adequate dilution from the sample to obtain concentrations from 5 to 25 mg L-1 for ondansetron and from 5 to 25 mg L^{-1} for midazolam admixture respectively. The same manner from the second mixture (0.5 mg mL^{-1} ondansetron-1.0 mg mL^{-1} midazolam), five standards solutions were prepared by adequate dilution from the sample to obtain concentrations from 2.5 to 12.5 mg L^{-1} for ondansetron and from 5 to 25 mg L^{-1} for midazolam admixture respectively. These standards solutions were divided into different aliquot parts, stored in Eppendorf tubes and frozen until each analysis day. All the procedures were done under aseptic conditions and using sterile drug solutions.

Instrument and chromatographic conditions: Mixtures concentrations were determined by a stability-indicating HPLC method. HPLC analysis was performed at room temperature (~25ºC) using a Shimadzu LC-6A pump equipped with Rheodine 7125 injection valve 20 µL, a Shimadzu SPD-6A spectrophotometric detector working at 254 nm. The signal from the detector was recorder and integrated with a chromatography data system Shimadzu C-R6A chromatopac; a LiChrospher ® 100 C18 (5 µm) LiChroCART® 250-4 column was employed. The mobile phase consisted of methanol:KH2PO4 0.05 M, adjusted to pH 3 with H3PO3 (60:40, v/v) delivered at flow rate of 1.0 mL min^{-1}. The sample injection volume was 20 µL, and triplicate injections were performed for every sample. The signal was recorded for ten minutes and the retention times were 4.1 min for ondansetron and 7.8 min for midazolam.

UV-vis spectrophotometer Cary 60 was also used for spectrophotometric measures at two wavelengths (250 and 310 nm).

Physical stability study: The physical stability of both drugs was determined by visual inspection of each sample control, with no colour change or appearance of turbidity initially. Visual inspection was done before of the determination ondansetron and midazolam concentrations by HPLC.

Chemical stability study: The initial concentration of mixture was defined as 100%, and subsequent sample concentrations were expressed as a percentage of the initial concentration. Stability of the mixture was defined as retention of at least 90% of the initial mixture concentration.

Forced degradation analysis: Forced degradation is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance [18-24].

In this work, five different studies were carried out for this purpose over each drug solution: acid, base, heat, UV light and hydrogen peroxide.

Results and Discussion

Accelerated degradation study

The subsequent studies were made over each drug solution containing 6 mg mL^{-1} of ondansetron or 10 mg mL^{-1} of midazolam.

pH study: To aliquot of 500 µL of each drug were added different amounts of HCl 1 M or NaOH 1 M (100, 250 and 500 µL). In the case of ondansetron, a new signal appears to 1.9 min when the HCl volume added was 250 or 500 µL being it higher when the volume and the time increases. On the other hand, the addition of NaOH about ondansetron signal gives two new signal (1.8 and 2.6 min). These signals enhanced with the volume of NaOH added and stay constant with the time. Additions of HCl over midazolam diminished the signal at the retention time and two new signals appear (about 3.1 min and 1.5 min) and increase with the volume of HCl added and with the time. Additions of NaOH diminished the signal at the retention time by dilution effect and a new signal appear at 2.5 min that enhanced with the volume of NaOH added and also remain stable with the time

Heat study: Two samples of each drug were heated at 40ºC and 60ºC during different times (from 10 to 60 minutes). No significant changes were observed in the chromatograms in all cases.

UV light: One sample of each drug was subject to UV irradiation during 24 h. The signal of the midazolam diminished with the time of exposition. With respect to the ondansetron no changes were observed.

Oxidants: To aliquots of 500 µL of each drug were added different amounts of H_2O_2 3% (100, 250, 500 µL). No effects were observed for midazolam while for ondansetron appears three new signals at 1.9, 2.8 and 3.6 min and whose areas enhanced with the volume of oxidant added.

Table 1: Statistical evaluation of data for different stability studies.

Ondansetron 0.5 mg mL^{-1} – Midazolam 1.0 mg mL^{-1} 37ºC and 25ºC							
Ondansetron (mg L^{-1})	**Mean**	**SD**	**RSD (%)**	**Minimum**	**Maximum**	**Confidence level**	
						Lower	**Upper**
2.5	47890	4062	8.48	41168	56118	45738	50042
5.0	100818	6617	6.56	86059	113686	98613	103023
7.5	150674	5127	3.40	133708	157699	148522	152826
10.0	202089	3362	1.66	192522	207415	199697	204480
12.5	254442	5023	1.97	240110	260804	252237	256647
Midazolam (mg L^{-1})	**Mean**	**SD**	**RSD (%)**	**Minimum**	**Maximum**	**Confidence level**	
						Lower	**Upper**
5	86795	8321	9.58	62347	100405	82266	913249
10	187848	15243	8.11	144693	210825	183423	192273
15	308672	11464	3.71	279963	322454	304031	313313
20	426153	8485	1.99	411940	446242	420964	431341
25	536856	13663	2.54	505774	558456	532095	541618
Ondansetron 0.1 mg mL^{-1} – Midazolam 0.1 mg mL^{-1} 37ºC and 25ºC							
Ondansetron (mg L^{-1})	**Mean**	**SD**	**RSD (%)**	**Minimum**	**Maximum**	**Confidence level**	
						Lower	**Upper**
5	91972	5051	5.49	81650	100945	84815	99130
10	185492	9312	5.02	169554	205661	177986	192999
15	275988	10576	3.83	257307	292206	268481	283494
20	388122	22230	5.73	358094	423456	380796	395448
25	466486	25392	5.44	426258	521388	459160	473812
Midazolam (mg L^{-1})	**Mean**	**SD**	**RSD (%)**	**Minimum**	**Maximum**	**Confidence level**	
						Lower	**Upper**
5	73554	6773	9.21	61716	89684	65435	81674
10	165463	6641	4.01	150851	178887	156904	174022
15	259574	12566	4.84	233661	281602	250767	268381
20	358434	20628	5.75	325798	399321	350510	366358
25	444816	27449	6.17	401995	497381	436696	452936

Physical stability study

All solutions were initially clear and colourless but visible particles appear into the infusers after 48 hours from its preparation.

Chemical stability study

HPLC method: The experimental data were processed making use of the Statgraphics Centurion XVI program [21]. Statistical evaluations of data for different stability studies are presented in Table 1 for each mixture. Calibration curves were linear over the concentration range used with acceptable correlation coefficients as can be seen in Table 2. The percentages remaining for

each mixture at two studied temperatures (25ºC and 37ºC) are shown in Figures 1 and 2.

UV-vis spectrophotometry method: The analysis of mixtures of absorbent substances is based on the fact that the total absorbance of a solution at a given wavelength is equal to the sum of the absorbances of the individual components present [22].

Table 2: Regression equations for admixtures.

Ondansetron-Midazolam Admixtures (mg mL^{-1})	T (ºC)	Drug	Method	Regression equation[21]
0.1 – 0.1	25ºC	Ondansetron	HPLC	Area = 17247.3 [Ondansetron] – 35908.6 R^2= 0.985
			UV-spectrophotometry	Equation (I) and (II)
		Midazolam	HPLC	Area (AU) = 14717.2 [Midazolam] – 47052.5 R^2= 0.994
			UV-spectrophotometry	Equation (I) and (II)
	37ºC	Ondansetron	HPLC	Area (AU) = 19033.1[Ondansetron] – 3884.6 R^2 = 0.998
			UV-spectrophotometry	Equation (I) and (II)
		Midazolam	HPLC	Area (AU) = 18709.9[Midazolam] -20279.5 R^2 = 0.9997
			UV-spectrophotometry	Equation (I) and (II)
0.5 – 1.0	25ºC	Ondansetron	HPLC	Area = 17951,6[Ondansetron] - 3361,6 R^2 = 0,998
			UV-spectrophotometry	Equation (I) and (II)
		Midazolam	HPLC	Area (AU) = 20958.8[Midazolam] -21031.4 R^2 = 0.9998
			UV-spectrophotometry	Equation (I) and (II)
	37ºC	Ondansetron	HPLC	Area (AU) = 20527.1 [Ondansetron] – 3501.6 R^2= 0.9997
			UV-spectrophotometry	Equation (I) and (II)
		Midazolam	HPLC	Area (AU) = 22428.3 [Midazolam] – 30391.9 R^2= 0.9994
			UV-spectrophotometry	Equation (I) and (II)

[a]*Regression equation obtained by Statgraphics Centurion XVI program*

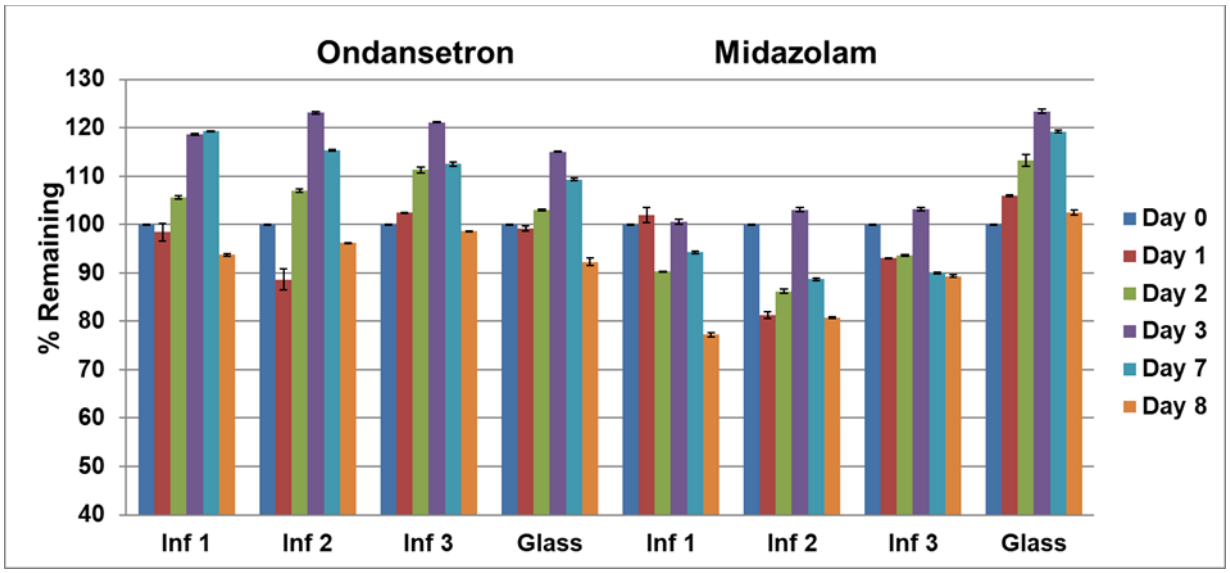
At first time we obtained the spectra for ondansetron and midazolam alone and mixture for obtained the measure's wavelengths (250 nm and 310 nm). At these two wavelengths the corresponding calibration curves for the two components are constructed for three consecutive days, calculating the corresponding mean values of both parameters for each component as shown

in the following equations. These equations were later used to obtain their concentrations:

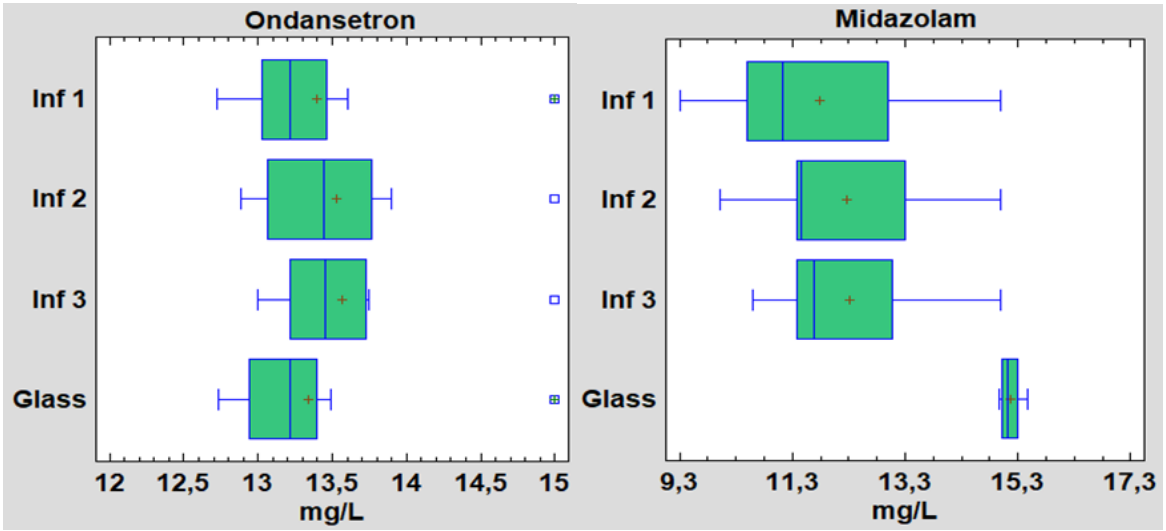
A_{250nm} = 0.0534[ondansetron] + 0.0444[midazolam] +0.2590 Equation (I)

A_{310nm} = 0.0490[ondansetron] + 0.0170[midazolam] + 0.2096 Equation (II)

HPLC-UV and UV-Vis spectrophotometric methods gave the same results respect to stability of the mixtures diluted in NaCl 0.9%: ondansetron-midazolam (0.1 mg/mL-0.1 mg/mL and 0.5 mg/mL-1.0 mg/mL) at two temperatures assayed are stable (retained >90% of their initial concentrations) only one day at 25ºC and 37ºC respectively as can be seen in the Figures 1 and 2. Ondansetron stored into infusers and glass was stable during all days assayed but midazolam is only stable into glass container at two temperatures and two concentrations studied.

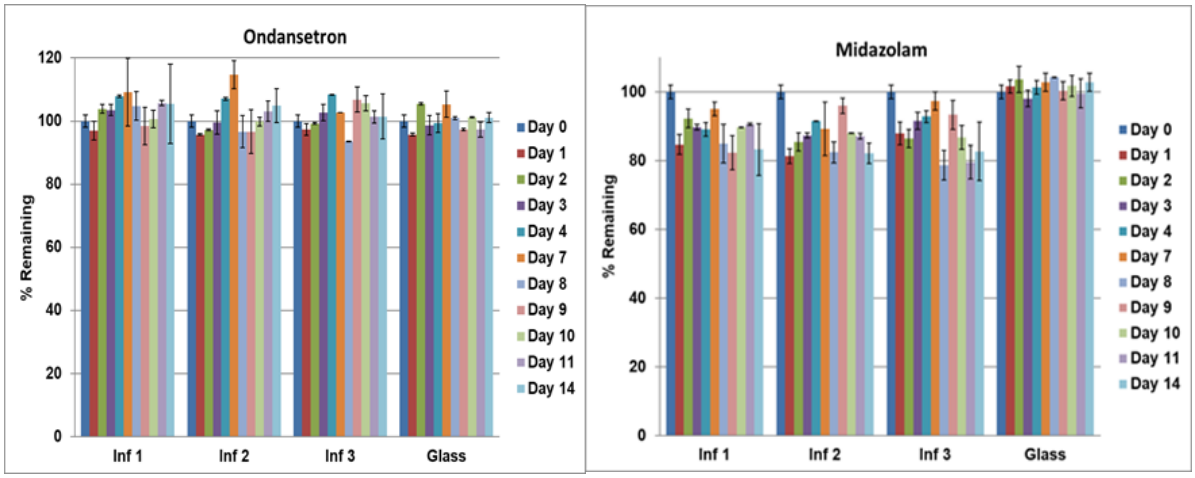


(**a**)

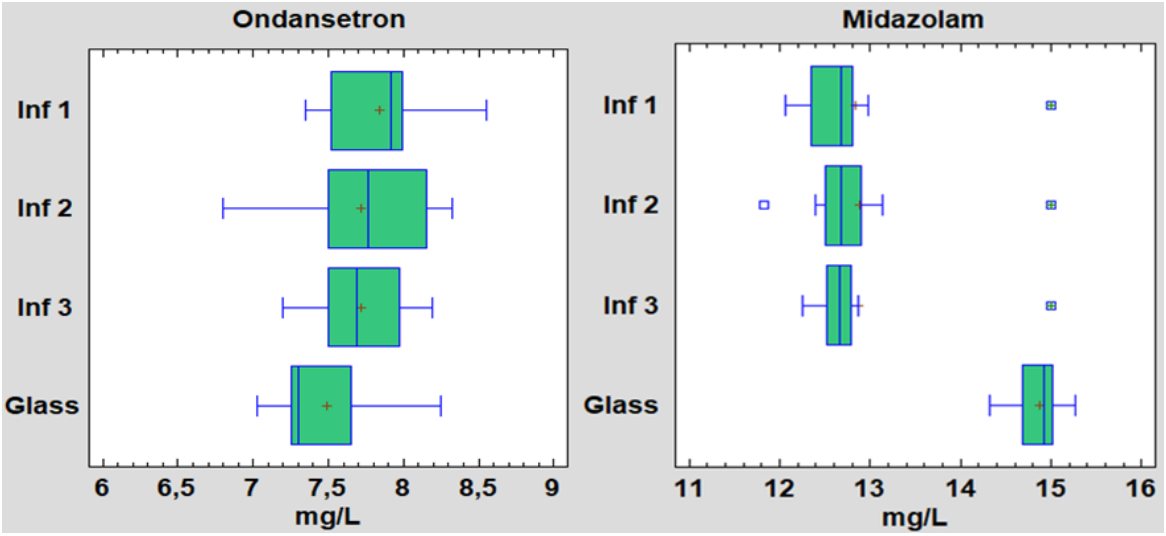


(**b**)

Figure 1: Percentages of mixtures remaining at 25ºC (0.1-0.1 mg/mL ondansetron-midazolam): (a) HPLC method (b) UV spectrophotometry method.



(**a**)



(**b**)

Figure 2: Percentages of mixtures remaining at 37ºC (0.5-1.0 mg/mL ondansetron-midazolam): a) HPLC method b) UV spectrophotometry method.

Conclusion

Two temperatures (25ºC and 37ºC) and two concentration levels have been assayed for ondansetron and midazolam admixtures, stored into infusers and glass all of their protected from the light. HPLC and UV-vis spectrophotometry methods have been used for stability studies, obtaining by both methods the same results. Therefore, it is suggested that admixtures can be stored in elastomeric infusers at the concentration ranges studied and may be used with confidence for 24 h after preparation. Over time ondansetron concentration remain stable but midazolam concentration diminished at 48 h after preparation as can be seen in Figures 1 and 2.

Infuser conditioning decreases stability with respect to other conditioning materials, so other stability studies may not be extrapolated if stored under different conditions.

Consent for Publication

Not applicable.

Conflict of Interest

The author declares no conflict of interest, financial or otherwise.

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