BRIEF REPORT

Stability in physiological saline of intravenous busulfan in a polyolefin pack

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Abstract

Introduction: Although it has been used orally, the variability in its absorption and the risk of causing vomiting has lead to a push towards the intravenous use of busulfan. This study looks at the stability of 60 mg of busulfan, in fixed volumes of 250 mL (0.24 mg/mL) and 500 mL (0.12 mg/mL) of physiological saline and different conservation conditions, in a new plastic pack made from polyolefin/polyamide laminates.

Material and methods: High-performance liquid chromatography with ultraviolet detection was used to determine the concentration of busulfan derivate with sodium diethyldithiocarbamate trihydrate. Stability was assessed for both concentrations; refrigerated and at room temperature, using the t_{90} of each sample.

Results: The percentage of the remaining busulfan concentration at 24 h was always less than 90%. At 25ºC and 0.24 mg/mL concentration, the t_{90} was 8.4 h; at 4ºC and a concentration of 0.24 mg/mL it was 16.7 h; at 25ºC and a concentration of 0.12 mg/mL it was 12 h and at 4ºC and a concentration of 0.12 mg/mL it was 11.5 h.

Conclusions: This study shows that busulfan in a concentration of 0.24 mg/mL in physiological saline is stable in the bags tested during a refrigerated storage period of 12 h plus 2 additional hours of administration of the drug.

Key words: Drug stability. Busulfan. Chromatography. High pressure liquid. Infusions. Intravenous.

Estabilidad en suero fisiológico del busulfán intravenoso en un envase de poliolefinas

Introducción: Aunque se ha utilizado por vía oral, la variabilidad en su absorción y el riesgo de que se produzcan vómitos, ha impulsado la utilización intravenosa de busulfán. En el presente trabajo se estudiará la estabilidad de 60 mg de busulfán, en volúmenes fijos de 250 ml (0,24 mg/ml) y 500 ml (0,12 mg/ml) de suero fisiológico y diferentes condiciones de conservación, en un nuevo envase de plástico, de lámina construida de poliolefina/poliamida.

Material y métodos: Se empleó la cromatografía líquida de alta eficacia con detección ultravioleta para determinar las concentraciones de busulfán derivatizado con dietilditiocarbamatotrihidrato sódico. La estabilidad se evaluó, para ambas concentraciones, tanto en nevera como a temperatura ambiente, mediante el t_{90} de cada ensayo.

Resultados: El porcentaje de concentración remanente de busulfán a las 24 h siempre fue inferior al 90%. A 25 ºC y concentración de 0,24 mg/ml el t_{90} fue de 8,4 h; a 4 ºC y concentración de 0,24 mg/ml fue de 16,7 h; a 25 ºC y concentración de 0,12 mg/ml fue de 12 h, y a 4 ºC y concentración de 0,12 mg/ml fue de 11,5 h.

Conclusiones: El presente estudio demuestra que el busulfán a una concentración de 0,24 mg/ml en suero fisiológico será estable en las bolsas ensayadas durante un período de almacenamiento de 12 h en nevera más las 2 h de administración del fármaco.

Palabras clave: Estabilidad del fármaco. Busulfán. Cromatografía. Líquido de alta presión. Intravenoso.

INTRODUCTION

Busulfan is a white or off-white powder with little solubility in water.[1] It functions as an electrophilic agent, acting specifically during the S phase of the cellular cycle. It reacts with nucleophilic atoms of the nucleic bases and forms inter and intrachain bridges in the DNA's double helix. This provokes interferences in DNA's transcription and replication process.[2]

In Spain busulfan recommendations, according to its technical data sheet,[3,4] vary if administered orally or intravenously. Oral recommendations are: in palliative treatment of the chronic phase of chronic granulocytic leukaemia; in prolonged remission of polycythaemia vera, especially in the case of marked thrombocytosis; and particular cases of essential thrombocythaemia, and myelofibrosis. Intravenous recommendations are: followed by cyclophosphamide, for treating previous conditioning to the transplant of haematopoietic progenitor cells in adult patients,

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when the combination is considered the most viable option; and followed by cyclophosphamide or melphalan, for treating previous conditioning to the transplant of haematopoietic progenitor cells in paediatric patients.

Although busulfan began to be used orally at high doses and combined with cyclophosphamide as a preparation means for bone marrow transplant, absence of adequate response, absorption variability and potential vomiting side effects encourage its intravenous use. The normal regimen for busulfan use is 0.8 mg/kg/6 h for 4 days intravenously.[5]

In the different pharmacopoeias reviewed, the only stability of busulfan tablets has limits between 93.0% and 107.0%, values which are usually used in tablets with active ingredients showing good stability characteristics. The absence of specification in these pharmacopoeias allows us to use this general classification for injectable doses with limits between 90.0% and 110.0%, limits which are used in pharmacopoeias for different injectable drugs such as brompheniramine or bumetanide.[6] Furthermore, this limit is used in injectable busulfan preparations which are discussed in previous studies.[3,7]

In Spain, intravenous busulfan (Busilvex®) shows a 6 mg/mL concentration in vials in a mix of N,N-dimethylacetamide and polyethylenglycol.[3] According to the manufacturer's instructions for intravenous administration, Busilvex® vials should be diluted in 5% physiological saline or glucose until reaching a concentration of 0.5 mg/mL. Stability at room temperature (25ºC) lasts up to 8 h[3,7] and while refrigerated at (2-8ºC), the mix remains stable up to 12 h.[3,7]

This study discusses the stability of a 60 mg (10 mL) standard dose of busulfan in fixed physiological saline volumes of 250 and 500 mL. These were obtained by the dilution of a commercial preparation subject to different storage conditions, in a new plastic pack made from polyolefin/polyamide laminates.

MATERIAL AND METHODS

Material

Busulfan Sigma® was used as the standard for validation of the chromatographic method and preparation of the calibration lines, and sodium diethyldithiocarbamate trihydrate (DDCT) Sigma® was used as the derivatizing agent. Acetonitrile Sigma-Aldrich® was used to prepare the busulfan solution, which preceded the mother solution preparation for calibrations. Other reagents used in preparation for the mobile phase were: Fresenius® bidistilled water and analytical grade Methanol (Merck)®.

Preparation of the busulfan intravenous mixtures was used with 500 and 250 mL sodium chloride bags with 0.9% Viaflo Baxter®.

Analytical technique

The technique used for determining busulfan concentrations was high-performance liquid chromatography (HPLC). The chromatography equipment used was a Spectra System AS1000 automatic injector, Waters 484 ultraviolet absorption detector, a Waters 515 pump and a Penelson NCI900 integrator connected to a computer.

For the stationary phase, a Kromasil® C18 column was used with a particle size of 5 µm and size of 150×4.6mm. For the mobile phase, a solution composed of methanol and water in an 80:20 proportion was used. The mobile phase was prepared by vacuum filtration through a 0.45 µm Millipore® membrane.

Elution flow of was 1.5 mL/min. Detection was done at a wavelength of 251 nm.

A busulfan standard mother solution of 25 µg/mL was prepared in physiological saline. 1.25 mg of busulfan was weighed and dissolved in 50 mL of acetonitrile to guarantee complete dissolving. Next, this mixture was diluted with 950 mL of physiological saline. Based on this mother solution, standard solutions were prepared in physiological saline (25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg/mL). 125 µL of derivatizing solution was added to each 500 µL calibrator (DDCT in bidistilled water at a concentration of 82 mg/mL[8]). Derivatized mixtures were shaken in Vortex® (Reax 1000) for 30 seconds at room temperature. 200 µL of derivatized mixture was injected into the chromatographic system by an automatic injector. This process was repeated 3 times for each standard dilution.

Accuracy of each calibrator was determined through relative error (Ev^i),[9] calculated as the quotient divided by the absolute error (Ea^i) ·x 100, expressed as the difference between the experimental value (Ve^i) and the interpolated value on the calibration line (Vi^i), and Vi^i $(Ev^i = [Ve^i \times Vi^i] \times 100 / Vi^i)$. Regarding precision, the references reviewed propose obtaining the coefficients of variation (CV^i)[10] calculated as the coefficient divided by standard deviation (SD^i) × 100 and the average of the 3 samples analyzed from each calibrator(X^i): $(SD^i \times 100)/X^i$. Linearity of the model is characterized by the determining coefficient (r^2).

Validation of the analytical method was carried out both intraday and interday, with the aim of ensuring that analysis always stayed within the recommended limits of accuracy and precision.[9]

Study of chemical stability

This study simulates actual preparation conditions of the intravenous mixture. Therefore the initial volume of intravenous fluid and quantity of medication in the pack as stated by the manufacturer are used. The intravenous mixture was prepared from busulfan vials (Busilvex® 60 mg/10 mL, Pierre Fabre Iberica Laboratory) by adding 10 mL from the preparation to the physiological saline bag, previously extracting 10 mL from the bag. 250 and 500 mL polyolefin bags were used (Viaflo®) which were kept at room temperature (25ºC) and refrigerated (4ºC). The final theoretical concentrations of busulfan intravenous mixtures were 0.24 and 0.12 mg/mL, respectively.

One mL samples were drawn from each bag at 1, 2, 4, 6, 8, 10, 12, and 24 h. After being drawn, these samples were diluted in physiological saline (1:40) and frozen at –40ºC until being

analyzed. Once thawed, these were processed in triplicate and just as described for standard solutions.

It was established that the busulfan degradation process corresponds to first order kinetics. To establish stability, t_{90} was determined through semilogarithmic regression of the percentage of the remaining busulfan concentration, in relation to time. By following recommendations from various international agencies,[11,12] the t_{90} of each sample was determined as the lower limit of the 95% confidence interval (CI), as it may indicate that in relation to time t_{90} the remaining busulfan concentration is >90%. This calculation was carried out by the Sigma Plot® computer program.

RESULTS

Analytical technique

In Figure 1, the chromatograms belonging to the highest concentration (25 µg/mL), the lowest (0.78 µg/mL) and the sample without busulfan (blank) of the calibration line are shown. The retention time of the resulting product from the derivatization process was 8.5 min. In Figure 1, there are no interferences in the retention time of busulfan.

Linearity is determined from the relationship between concentrations and the chromatogram areas of the model and is characterized by an ordinate regression line in the origin –82 938 (95% CI, –112 456 to –53 419) and unresolved 141 618 772 (95% CI, 193 113 724 to 144 123 821) and whose determining coefficient was >0.999 in sample concentrations. Relative error and CV were less than 10%.

Sensitivity of the method was 0.635 µg/mL, limit of detection 1.836 µg/mL and limit of quantification 6.119 µg/mL.

Chemical stability

In Table 1, the average percentages of busulfan remaining in the stored intravenous mixtures at room temperature and in refrigeration at different sampling times are shown. In all conditions tested, the percentage of remaining busulfan concentration at 24 h was <90%.

In Figure 2, busulfan degradation curves are shown at different temperatures and preservation conditions, such as its regression parameters and its t_{90}. At 25ºC and a concentration of 0.24 mg/mL the t_{90} was 8.4 h; at 4ºC and a concentration of 0.24 mg/mL it was 16.7 h; at 25ºC and a concentration of 0.12 mg/mL it was 12 h, and at 4ºC and 0.12mg/ml it was 11.5 h.

DISCUSSION

The analytical technique used for determining busulfan allows the peak of the remaining product from the derivatization process of sample impurities to be adequately separated, and it shows adequate stability, accuracy and precision.

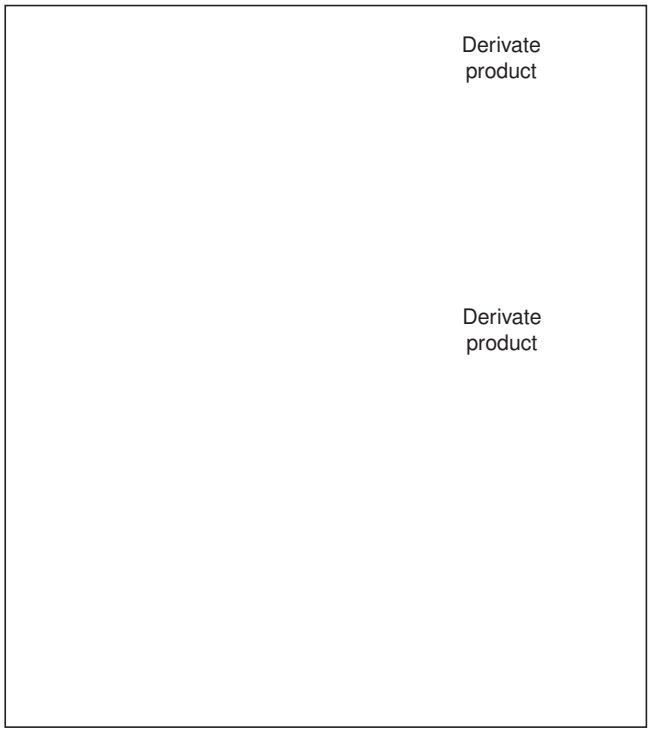

Figure 1. *Chromatograms corresponding to the concentration of 25 µg/mL (A) and 0.78 µg/mL (B) of busulfan, and the blank sample without busulfan (C).*

This study has established busulfan stability in intravenous mixtures in polyolefin bags at 2 concentrations, 0.24 and 0.12 mg/mL, stored in refrigeration and at room temperature.

During the 24 h of the study, the remaining concentrations of busulfan were higher than the limits of quantification of the analytical technique, and this confirmed validity of the concentrations recorded.

The 0.24 and 0.12 mg/mL concentration bags kept at room temperature for 24 h lost 10% of their concentration (t_{90}) at 8.4 and 12.3 h, respectively. These values coincide with those published in the references regarding busulfan stability.[3,7] Also, bags kept in refrigeration at 4ºC for 24 h lost 10% of their concentration (t_{90}), the 0.24 mg/mL bag at 16.7 h, and the 0.12 mg/mL bag at 11.5 h.

The data obtained coincide with the manufacturer's instructions on the technical data sheet[3] and distinguished stability treaties.[7] However, this is distinct from data presented in the Karstens y Krämer[13] article, which concludes a much higher stability up to 48 h, in 0.5 mg/mL physiological saline solutions maintained at 13-15ºC. Nevertheless, it should be noted that in this study, the t_{90} is calculated by directly interpolating on the regression line and not on the line which marks the lower limit of the 95% CI. This limitation is indicated in the article published by Gaisford et al,[14] who obtain stability in a busulfan vial diluted in 50 mL of physiological saline for 27 h, between 24 and 6ºC plus 3 h at 25ºC.

Table 1. Average percentages (%) of remaining busulfan intravenous mixtures kept at room temperature and in refrigeration at different sampling times (n=3)

Time, h	Busulfan (0.24 mg/mL)				Busulfan (0.12 mg/mL)			
	25 ºC		4 ºC		25 ºC		4 ºC	
	Average, %	SD, %	Average, %	SD, %	Average, %	SD, %	Average, %	SD, %
1	100.4	0.078	100	2.131	100	2.761	100	1.27
2	99.1	2.768	99.1	0.365	101.7	1.913	98.3	0
4	97.8	0.908	98.3	2.141	97.4	2.061	94.1	4.565
6	93.6	0.55	94.1	2.453	98.2	3.268	95	5.726
8	89.8	1.139	94.5	0.801	93.1	0.217	97.5	4.557
10	87.7	3.338	94.1	0.766	93.1	3.463	89.1	5.97
12	85.2	1.89	95.3	1.794	90.5	2.215	90.8	9.32
24	75.5	3.643	88.2	2.173	80.3	2.054	85	1.155

SD indicates standard deviation.

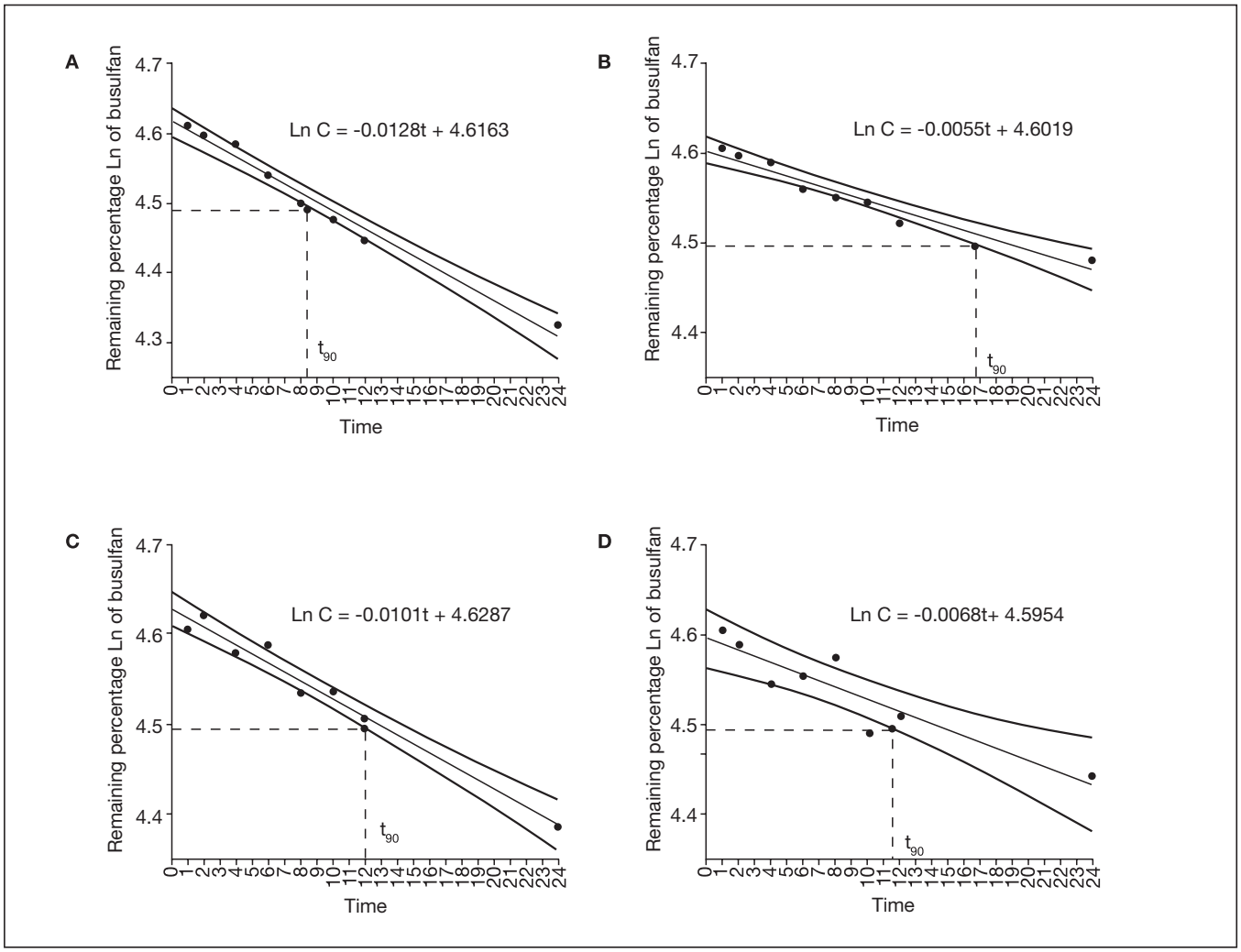

Figure 2. *The regression line and confidence interval (CI) of the napierian logarithm of the percentage of remaining busulfan in relation to time, for different concentrations and conditions of preservation. A) t_{90} 25ºC 0.24 mg/mL; B) t_{90} 4ºC 0.2 4mg/mL; C) t_{90} 25ºC 0.12 mg/mL; D) t_{90} 25ºC 0.12 mg/mL.*

In conclusion, this study shows that a 0.24 mg/mL concentration of busulfan in physiological saline is stable in sample bags for a storage period of 12 h in refrigeration plus 2 h for the drug's administration.

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