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HPLC and GLC determination of residual solvents in busulphan ☆

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Abstract

Quantitative methods using HPLC and GLC for the determination of residual pyridine and ethanol in busulphan have been developed. The measurement errors for pyridine and ethanol in busulphan samples ($p = 0.95$) were found to be 10% and 7%. It was shown that one recrystallization enabled pure busulphan to be prepared.

Keywords: Busulphan; Ethanol; Pyridine; HPLC; GLC

1. Introduction

Busulphan is widely used in medical practice. The synthesis of busulphan is carried out in pyridine, which can remain in the final product as a residue. Traces of ethanol can also remain even after recrystallization of the product. A method for the analytical determination of pyridine and ethanol in busulphan is required.

Pyridine is a nitrogenous basic substance [1]. This and related substances can be analysed by HPLC using Zorbax ODS [2], Ultrasphere ODS [3], Aminex [4] with complex eluents contained buffer systems. However, adequate control methods for trace quantities of admixtures of pyridine and ethanol in busulphan have not been reported in the literature.

The purpose of this work was to select appropriate conditons for the quantitative and qualitative analysis and to elaborate methods for the control of trace quantities of ethanol and toxic pyridine remaining as admixtures in the final product busulphan.

2. Experimental

2.1. Calibration solutions of pyridine

Calibration solutions of pyridine were prepared by dissolving 2 mg of the compound in 20 ml of eluent. From the initial solution, working pyridine solutions were prepared by diluting with eluent

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50, 500 and 1000 times. To each of five flasks containing 0.1 g of busulphan was added 2 ml of each dilvent solution, carefully mixed, filtered and 20 µl was injected into the HPLC column for analysis.

2.2. Calibration solutions of ethanol

Calibration solutions of ethanol were prepared by dissolving 1 mg of the compound in 2 ml of distilled water. From the initial solution, working ethanol solutions were prepared by diluting with water 10, 25, 50, 100 and 1000 times. To each of five flasks containing 0.1 g busulphan was added 2 ml of each diluent, carefully mixed, filtered and 20 µl was injected into the GLC column for analysis.

2.3. Extraction of pyridine and ethanol from busulphan samples

0.1 g of the sample of busulphan was put in a flask and 2 ml of distilled water was added, carefully mixed, and the mixture was filtered. 20 µl of the filtrate was subjected to analysis.

2.4. Quantitative analysis

Quantitative analysis was carried out by the absolute calibration method. The pyridine (ethanol) concentration in the busulphan sample was calculated from the equation:

$$X = \frac{C_{st} G}{m}$$

where X is the pyridine (ethanol) concentration in the busulphan sample (%w/v); C_{st} is pyridine (ethanol) concentration in the extract of busulphan sample (%w/v); G is the weight of extract of busulphan sample (g) and m is the weight of busulphan sample (g).

The main substance busulphan did not allow direct pyridine and ethanol determination because of slight solubility in eluent and slight absorption at about λ 254 nm.

2.5. Analytical conditions

HPLC of pyridine

Analysis conditions for pyridine were: a glass column (150 × 3.3 mm i.d.); Separon SGX C_{18} with acetonitrile–phosphate buffer (pH 6.7; 0.025 M) (90:20, v/v); Separon SGX NH_2 with: (a) acetonitrile–phosphate buffer (pH 6.7; 0.025 M) (90:20, v/v); (b) acetonitrile–dibutylammonium phosphate buffer (pH 7.0; 0.8 mM) (90:20, v/v); (c) acetonitrile–dibutylammonium phosphate buffer (pH 7.8; 1.25 mM) (90:20, v/v). Flow-rate 1 ml min^{-1}; λ 254 nm; the temperature was ambient. Double-distilled water was used. Busulphan samples were prepared by the method described in Ref. [5], recrystallized with ethanol and dried under vacuum at 6.7 kPa (30 °C) for 30 min.

GLC of ethanol

Analysis conditions for ethanol were: a stainless-steel column (1.2 × 3 mm i.d.); Polysorb-1; the column temperature was 100 °C, the carrier gas was helium at a flow-rate of 30 ml min^{-1}; a flame ionization detector was used.

Table 1
Retention times and capacity factors for pyridine and ethanol

Eluent	Separon SGX NH_2		Separon SGX C_{18}		Polysorb-1	
	t_r (min)	k'	t_r (min)	k'	t_r (min)	k'
Acetonitrile–phosphate buffer (pH 6.7; 0.025 M) (90:20, v/v)	3.0	1.25	2.06	0.67		
Acetonitrile–dibutylammonium phosphate buffer (pH 7.0; 0.8 mM) (90:20, v/v)	4.0	1.37	2.33	0.70		
Acetonitrile–dibutylammonium phosphate buffer (pH 7.8; 1.25 mM) (90:20, v/v)	4.5	3.8	2.20	0.86		
Helium					2.0	5.5

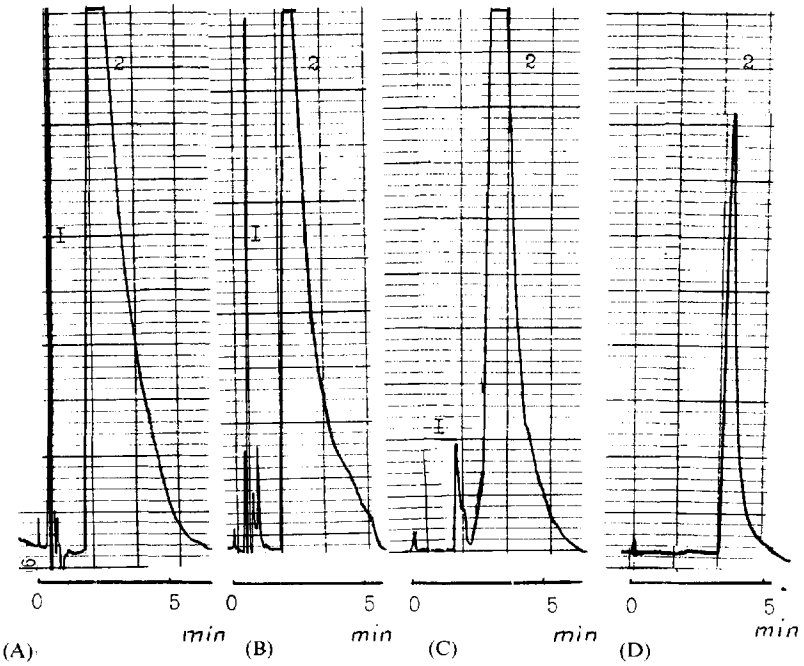

Fig. 1. Chromatograms of pyridine extracts from busulphan samples obtained using HPLC with different eluents. A, Separon SGX C_{18} and B, Separon SGX NH_2 with acetonitrile–phosphate buffer (pH 6.7;025 M) (90:20, v/v); Separon SGX NH_2 with acetonitrile–dibutylammonium phosphate buffer (pH 7.0; 0.8 mM):(90:20, v/v); Separon SGX NH_2 with acetonitrile–dibutylammonium phosphate buffer (pH 7.8; 1.25 mM) (90:20, v/v). 1, Solvent; 2, pyridine.

2.6. Validation procedure

Five solutions containing 0.01 (0.05), 0.0002 (0.002), 0.0001 (0.001), 0.00001 (0.0005), 0.000002 (0.00005)%w/v of pyridine (ethanol) were prepared in five flasks with 20 ml of eluent (2 ml of water) containing 0.1 g busulphan (busulphan extracts).

Five replicate samples from each concentration were extracted and analysed. Concentrations were determined by comparison with a calibration curve. A weighted linear-regression method used

Table 2
Accuracy and precision data for percentage concentration of pyridine and ethanol[a] obtained from back-calculated standard curve concentrations

Pyridine, %w/v (Ethanol, %w/v)	1×10^{-2} (5×10^{-2})	2×10^{-4} (2×10^{-3})	1×10^{-4} (1×10^{-3})	1×10^{-5} (5×10^{-4})	2×10^{-6} (5×10^{-5})
Mean	1.01×10^{-2} (5.06×10^{-2})	2.2×10^{-4} (2.07×10^{-3})	1.1×10^{-4} (1.99×10^{-3})	1.05×10^{-5} (5.3×10^{-4})	2.2×10^{-6} (5.19×10^{-5})
SD	0.053×10^{-2} (0.354×10^{-2})	0.161×10^{-4} (0.166×10^{-3})	0.076×10^{-4} (0.052×10^{-3})	0.083×10^{-5} (0.490×10^{-4})	0.215×10^{-6} (0.466×10^{-5})
RSD (%)	5.248 (6.996)	7.318 (8.019)	6.909 (4.771)	7.905 (9.245)	9.772 (8.979)
Accuracy	101.0 (101.2)	110.0 (103.5)	110.0 (109.0)	105.0 (106.0)	110.0 (103.8)

[a] Data for ethanol is indicated in parentheses.

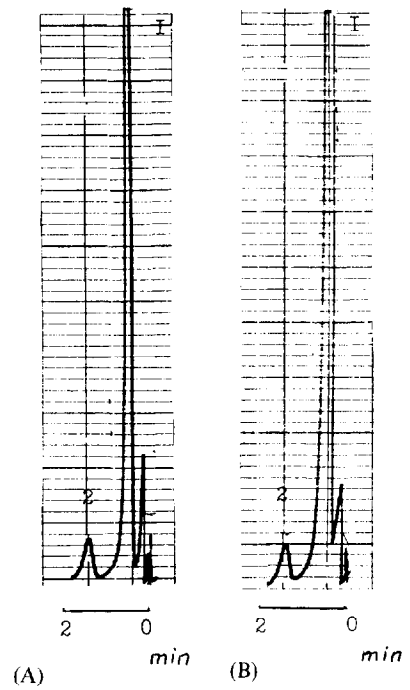

Fig. 2. Chromatograms of standard solution with 10^{-2}%w/v ethanol concentration (A) and extract of the busulphan sample M-5 (B) using GLC. Polysorb-1; carrier gas, helium. 1, Solvent; 2, ethanol.

Table 3
Determination of pyridine and ethanol in busulphan

Sample	Residual concentration in busulphan, % w/v	
	Pyridine, $\times 10^{-4}$	Ethanol, $\times 10^{-2}$
M-1	2.0	0.4
M-5	0.26	7.0
M-6	1.0	8.0
M-8	0.4	0.4
M-9	0.53	3.0

to construct a calibration curve for the peak-area ratio of analyte to internal standard versus analyte concentration.

From the data the precision (determined as the RSD of the ean) and the mean accuracy were determined (Table 2).

The recovery of pyridine and ethanol and the internal standards from busulphan extracts was estimated with five determinations by comparing the peak areas obtained with processed samples to those obtained by direct injection of known amounts of the compounds, equivalent to 100% recovery. The recovery of pyridine (ethanol) at busulphan extract concentrations of 0.01 (0.005) and 2×10^{-6} (5×10^{-5})%w/v was $95.5 \pm 5.25\%$ (97.2 ± 7.4) and $92.8 \pm 9.3\%$ (94.6 ± 8.7) respectively. Correlation coefficients obained using weighted ($1/Y$) linear regression analysis of calibration curves were typically 0.999.

3. Results and discussion

Chromatograms of pyridine extracts and ethanol extracts from busulphan samples are shown in Figs. 1 and 2, respectively. With Separon SGX C_{18} and acetonitrile–phosphate buffer (pH 6.7; 0.025 M) (90:20, v/v) low values of capacity factor k' were obtained and tailing for the pyridine peak was observed (Fig. 1A and Table 1). On replacement of Separon SGX C_{18} with Separon SGX NH_2, the capacity factor k' increased and the peak shape improved (Figs. 1B, 1C and 1D). The nature of the buffer solution influenced the capacity factor k' and the peak symmetry. Dibutylammonium phosphate was more effective than phosphate buffer. According to the results presented in Table 1, the eluent of acetonitrile–dibutylammonium phosphate buffer provided the best capacity factor k' for pyridine.

One recrystallization enabled the preparation of pure samples with a pyridine concentration of 0.2×10^{-4}–2.0×10^{-4}%w/v (Table 3). Ethanol concentrations in busulphan samples were 0.004–0.02%w/v (Table 3).

4. Conclusions

The effect of different analytical conditions on the determination of pyridine in busulphan extracts was studied. Methods for the analytical control of pyridine and ethanol admixtures in busulphan using HPLC and GLC were developed. It was shown that one recrystallization allowed pure busulphan samples to be prepared.

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