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The determination of busulphan in dissolution samples by flow injection analysis

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Abstract

A robust colourimetric flow injection analysis (FIA) procedure is described for the determination of busulphan in dissolution samples of a 2 mg tablet formulation. The sample solution is injected directly into a reagent stream containing 4-(4-nitrobenzyl)pyridine/potassium hydrogen phthalate. An on-line heating stage allows the formation of a coloured pyrridinium salt species, which following stabilisation is detected spectrophotometrically at 570 nm. The method has been fully validated and is linear over the concentration range 0.004–0.024 mg of busulphan ml^{-1}. The method can also been applied to uniformity of content and bulk assay testing. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Flow injection analysis; Automation; Busulphan; Myleran®; Dissolution

1. Introduction

Busulphan is an alkylating agent and is well established for the palliative treatment of chronic myelocytic leukaemia and the management of polycythemia vera. The drug is administered as a tablet formulation, typically using 2 mg tablets. Dissolution testing is required for routine control of the rate of release of busulphan from the tablet formulation, as the active principle is highly potent.

The British Pharmacopeia (BP) monograph for busulphan tablets involves a non-specific titrimetric assay method, which lacks the necessary sensitivity for monitoring dissolution solutions. As a result, there is no dissolution test for busulphan tablets in the BP monograph. A similar situation prevails in the US Pharmacopeia.

This work describes the development and validation of a rapid method based upon an established reaction between alkylating agents and 4-(4-nitrobenzyl)pyridine and employing flow injection analysis (FIA) [1]. An earlier paper [2] describes the application of FIA to the analysis of busulphan, among other alkylating anti-tumour drugs, in acetone solution down to a concentration of 0.5 mg ml^{-1}.

In this paper the approach is applied wholly to aqueous solutions (as obtained in dissolution test-

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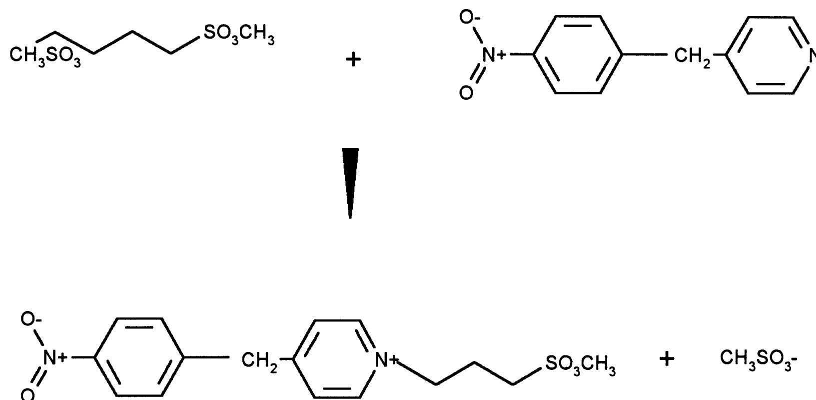


Fig. 1. Reaction between busulphan and 4-(4-nitrobenzyl)pyridine.

ing), at concentrations down to 0.004 mg of busulphan ml^{-1}.

Busulphan reacts with 4-(4-nitrobenzyl)pyridine, at elevated temperature. Reaction details are given in Fig. 1.

The resultant pyrridinium salt, which under the conditions of the method is proportional to the amount of busulphan present, is determined spectrophotometrically at 570 nm. The pyrridinium salt is a transient species, and is stabilised prior to detection by mixing with a solution of piperidine in ethanol. Water produces a significant, but reproducible blank response, which is subtracted from sample and standard peaks prior to the calculation of results. Rapid analysis times offset the problems associated with busulphan's poor aqueous stability.

2. Experimental

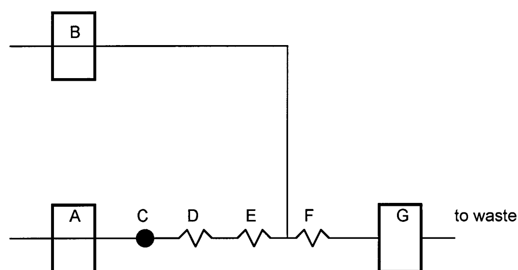
2.1. Apparatus

The flow injection manifold is shown schematically in Fig. 2.

The work was carried out using a Gilson 231/401 autosampler, a Waters 600E HPLC pump, a Gilson 307 HPLC pump, a Kratos Spectroflow 757 UV detector fitted with a tungsten lamp, a Shimadzu CTO-6A column oven (later replaced with Gallenkamp vacuum oven) and a Techne RB-5 refrigerated bath. Stainless steel (0.5 mm i.d.) was used for all connections and reaction tubing.

2.2. Flow injection analysis conditions

Reagent stream 1 was a solution consisting of 2% (w/v) 4-(4-nitrobenzyl)pyridine and 3% (w/v) potassium hydrogen phthalate in ethylene glycol: water (70:30 v/v), pumped at a constant flow rate of 1.4 ml min^{-1}. Reagent stream 2 was piperidine: ethanol (50:50 v/v), pumped at a constant flow rate of 0.6 ml min^{-1}.



A: Reagent 1, at 1.4 mL/minute
B: Reagent 2, at 0.6 mL/minute
C: Injection valve - 200uL
D: Reaction coil 17m., 140 C
E: Cooling coil 1m., room temperature
F: Mixing coil 3m., room temperature
G: Detection at 570nm

Fig. 2. Flow injection manifold for FIA system.

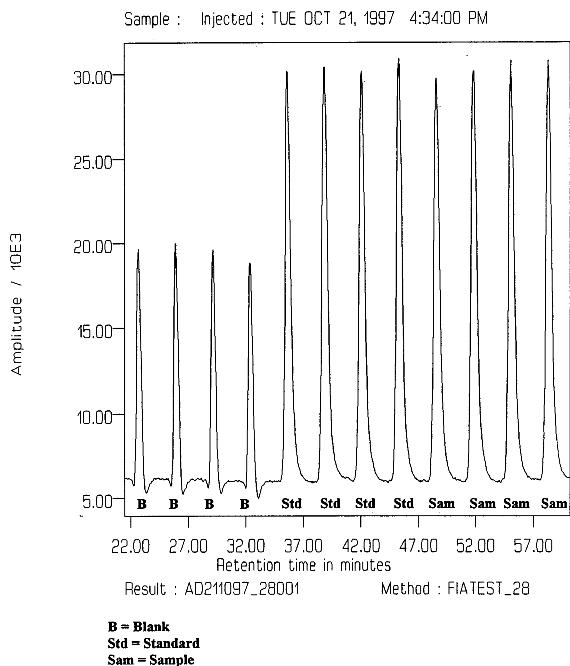


Fig. 3. Typical FIA signal outputs for standard, sample and blank solutions.

The reaction tubing was a 17 m length of 0.5 mm i.d. stainless steel tubing (coiling diameter 20 cm), heated to 140°C. The cooling coil was a 1 m length of 0.5 mm i.d. stainless steel tubing (coiling diameter 4 cm), cooled with water at room temperature The mixing coil was a 3 m length of 0.5 mm i.d. stainless steel tubing (coiling diameter 5 cm). The injection volume was 200 μl. A detection wavelength of 570 nm was employed, with a sensitivity of 0.1 a.u.f.s.

Table 1
Selectivity of the FIA assay condition to potential impurities

Compound name	Impact on blank response
Methanesulphonic acid	No significant change to response
Butan-1,4-diol	No significant change to response
Tetrahydrofuran	No significant change to response
Unstressed placebo tablets	2–3% Increase to response
Placebo tablets stored for 17 days at 50°C/ambient RH	2–3% Increase to response

2.3. Reagents

All reagents were of analytical grade and obtained from Sigma Aldrich (Poole, Dorset, UK).

2.4. Sample solution

Four Myleran® tablets 2 mg (GlaxoWellcome, Temple Hill, Dartford, UK) were introduced into a USP apparatus II dissolution vessel containing 500 ml of water at 37°C, paddle speed 50 rpm. An aliquot was removed at the appropriate sampling time and centrifuged at 3000 rpm for 5 min. The clear supernatant was the sample solution. All solutions were stored at ≈ 4°C prior to injection, solutions have a shelf-life of 24 h under these conditions.

2.5. Standard solution

A suitable batch of busulphan drug substance was selected as the standard material. An accurately weighed amount of busulphan (≈ 16 mg) was transferred to a 100 ml volumetric flask, 5.0 ml of acetonitrile were added and the flask was shaken until complete dissolution occurred. The flask was then diluted to volume with water and mixed well. 10.0 ml of this solution were transferred to a 100 ml volumetric flask and then diluted to volume with water to give the standard solution. All solutions were stored at ≈ 4°C prior to injection, solutions have a shelf-life of 24 h under these conditions.

2.6. Blank solution

Water was used as the blank solution.

2.7. Procedure

Reagent streams 1 and 2 were pumped through the manifold until a stable baseline was obtained.

Portions of the blank solution were injected until the reproducibility of the peak areas was satisfactory (RSD by area < 3.0%). The standard solutions were then injected followed by the sample solutions. The analysis was concluded with bracketing standard injections. The mean blank response was then subtracted from all standard and sample solutions. By comparison of the corrected peak areas of the samples and bracketing standards, the percentage of busulphan dissolved was calculated.

3. Results and discussion

3.1. Method development

A variety of techniques were investigated as possible replacements for an existing Technicon Autoanalyzer method (which was also based on the reaction with 4-(4-nitrobenzyl)pyridine) for determining busulphan in dissolution samples. This method required replacing as it was slow, gave poor reproducibility and utilised redundant equipment.

Method development was complicated by busulphan's lack of a chromaphore and poor aqueous stability. Methods investigated included HPLC with either UV, refractive index and electrochemical detection and CE with indirect UV detection. In addition a literature search revealed direct GC injection to be impractical. None of these options proved suitable and it was decided to investigate development of an FIA method.

Initial development work attempted to reproduce the work of an earlier paper [2], with a view to greatly improving the sensitivity and extending the application to aqueous solution. As expected peak size was quickly found to be proportional to reaction coil length, temperature and injection volume. Due to the large difference in viscosity between reagent streams 1 and 2, a very noisy baseline was obtained.

Initially a Shimadzu CTO-6A column oven was used to heat the reaction coil, with a maximum operating temperature of 99°C. Due to incomplete reaction, peaks produced at this temperature were found to be too small to reproducibly integrate against the baseline noise. Efforts were therefore concentrated on reducing this noise. A marked improvement in the baseline was attained by introducing 20% v/v ethanol into the reagent stream 1 to reduce its viscosity.

As dissolution samples would be prepared in aqueous media, water was injected onto the system to check for a potential blank response. Water was found to produce peaks under these conditions. To eliminate these peaks varying percentages of water were introduced into the ethylene glycol stream, the expectation being that the blank response would be masked by the presence of water in the reagent stream. This approach was unsuccessful, however a composition of 70:30 v/v ethylene glycol:water gave a considerable improvement in baseline noise. Further increases to the water content of reagent stream 1 were avoided due to the likelihood of reagent precipitation.

With reduced baseline noise, work switched to increasing peak size. Reaction coil length was progressively increased from a starting length of 10 to 17 m and peak size was found to be proportional to reaction coil length. However, the corresponding time from injection to detection also increased as did the resultant back pressure and peak width therefore further increases in the reaction coil length above 17 m were not pursued.

Peaks were still too small to allow the accurate quantification of dilute dissolution samples. Attention then turned to studying peak area as a function of reaction temperature. The Shimadzu HPLC column oven was replaced with a Gallenkamp oven, allowing temperatures of 200°C to be attained. The operating temperature was incrementally increased, in ≈10°C steps, from 99°C. Each increase was found to approximately double the resulting peak area, up to a temperature of 130°C. However, an increase in reaction temperature from 130 to 140°C produced only a slight increase in peak size. This strongly suggested that complete reaction of the busulphan sample was occurring at this elevated temperature. Increasing the injection volume to 200 μl resulted in much larger peaks. At this

stage the peak size was considered sufficient to allow quantification of dissolution samples.

The one remaining problem was the peak shape. Peaks were ≈ 2 min broad and showed a tendency to split at the apex. Increasing the flowrates of the two streams (while maintaining the ratios) sharpened the peaks and reduced the analysis time, while lengthening the mixing column from 2 to 3 m eliminated the peak splitting. Under these conditions the blank response was found to be reproducible and subtractable. The limit of detection was satisfactory. Typical FIA signal outputs for standard, sample and blank solutions are shown in Fig. 3.

3.2. Validation

Validation was carried out specifically for the determination of busulphan in Myleran® tablets 2 mg. Assuming no inert interference, the method should be applicable to other busulphan formulations.

3.3. Linearity of the method

The linearity of the method over the concentration range 0.0039–0.0238 mg of busulphan ml^{-1} (equivalent to 0–150% of the nominal assay concentration of 0.016 mg ml^{-1}) was confirmed by injecting a series of standard solutions at levels of 0, 25, 50, 75, 100, 125, and 150% of the nominal concentration. A linear response of peak area (corrected for blank response) versus concentration was obtained: $y = 61380344 \times -55957.4$ (correlation coefficient $= 0.9992$). Intercept was found to be equivalent to -6.0% of the peak area of a standard solution at nominal concentration.

3.4. Specificity

Aqueous solutions of several manufacturing impurities and degradation products of busulphan were examined by the method. The response obtained for these solutions was compared to that of the blank. No significant changes in response were detected for the impurities injected. An inert formulation (omitting only the busulphan) was also examined by the method. An increase in response equivalent to 2–3% of the area of a standard solution was obtained. This level of interference is considered acceptable. The method was judged to be specific in the presence of other components of Myleran® tablets. (Table 1).

3.5. Accuracy

The accuracy of the method was determined by recovery experiments. In triplicate experiments, known quantities of busulphan were added to an inert formulation at amounts equivalent to 50, 100, and 150% of the nominal content. These samples were then tested by the method. Recoveries of 97.5, 96.9, and 98.7% of the added amount for the addition of 50% of nominal concentration; 99.6, 100.6 and 101.9% of the added amount for the addition of 100% of nominal concentration; and 98.7, 99.0 and 100.2% of the added amount for the addition of 150% of nominal concentration were found.

3.6. Precision

The repeatability of the method was assessed by carrying out six injections of a standard solution on a single occasion. A RSD of 0.53% was obtained for the peak area (corrected for blank). Additionally the repeatability of the standard preparation was investigated by preparing and running six standard solutions on a single occasion. A RSD of 1.03% was obtained for the mean response factor (corrected for blank).

3.7. Robustness

Typically, the reagents employed have been prepared with limits of $\pm 10\%$ of the nominal composition, with no adverse affect. As a blank response was obtained from water it is important to match the sample and standard matrices as closely as possible.

4. Conclusions

A robust and sensitive FIA procedure has been

developed to allow rapid and automatable determination of the busulphan content of dissolution samples. The method has been validated for a 2 mg tablet formulation and is more sensitive and stability indicating than current procedures. Comparative testing of batches of tablets has demonstrated equivalent results. The method can also be applied to bulk assay and content uniformity testing where sample solutions are prepared in aqueous media.

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References

[1] A.M. Agree, R.L. Meeker, Talanta 13 (1966) 1151–1160.
[2] M. Suzuki, T. Nishinaka, S. Takitani, Iyakuhin Kenkyu 18 (5) (1987) 749–752.

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