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The determination of methanesulphonic acid content of busulfan drug substance and busulfan (Myleran®) tablets by ion chromatography

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Abstract

A robust ion chromatographic procedure is described for the determination of the methanesulphonic acid content of busulfan drug substance and busulfan (Myleran®) 2 mg tablets. The sample was dissolved in aqueous acetonitrile, butanesulphonic acid was added as an internal standard and the solution was injected onto an ion selective column, with ion suppression and conductivity detection. The method has been fully validated and is linear over the concentration range 0.295–14.73 μg of methanesulphonic acid/ml. The method has been demonstrated to be stability indicating. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ion chromatography; Busulfan; Myleran® tablets; Methanesulphonic acid

1. Introduction

Busulfan is an alkylating agent and an antileukaemic and is well established for the palliative treatment of chronic myelocytic leukaemia and polycythemia vera. The drug is administered as a tablet formulation, typically at the dosage level of 2 mg per tablet. Methanesulphonic acid is formed by hydrolysis of busulfan, therefore the monitoring of methanesulphonic acid levels gives an accurate indication of the stability of busulfan. A selective accurate procedure is therefore required for routine control of the levels of methanesulphonic acid in busulfan tablets and drug substance.

This work describes the development and validation of an ion chromatographic method for determination of methanesulphonic acid content.

2. Experimental

2.1. Method development

A literature search revealed an existing ion chromatographic method and conditions for the analysis of methanesulphonic acid, in use by Quality Assurance, Glaxo Wellcome Operations, Dartford [1,2].

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In this method water was used as the dissolving solvent. The method was modified to include acetonitrile in the dissolving solvent, to aid in dissolving and to reduce decomposition of busulfan.

An internal standard, 1-butanesulphonic acid, was also added to both the standard and sample solutions. Validation experiments were conducted on this method.

2.2. Apparatus

The work was carried out using a Dionex 2000i/SP ion chromatograph conductivity detector, a Gilson 231/401 autosampler, an LDC Constametric III HPLC pump, and a Shimadzu CTO-6A column oven. Tefzel and PTFE tubing was used for most connections, with a stainless steel Rheodyne loop used for the autosampler injector.

2.3. Chromatographic conditions

The eluent was a solution consisting of aqueous sodium hydrogen carbonate (0.014% w/v), pumped at a constant flow rate of 2 ml/min. The suppressant was aqueous sulphuric acid (0.075% v/v) pumped at a constant flow rate of 3 ml/min.

The detector sensitivity was 100 μs, the injection volume was 50 μl and the temperature was controlled at 30°C.

2.4. Reagents

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

A mixture of acetonitrile:water (30:70 v/v) was used as the dissolving solvent.

The internal standard solution was prepared by accurately weighing 75 mg of 1-butanesulphonic acid and sodium salt, into a 1000 ml volumetric flask, dissolving in and diluting to volume with dissolving solvent.

2.5. Sample solution

For the drug substance, 3 mg of busulfan was accurately weighed into a suitable vessel, 1 ml of internal standard solution and 9 ml of dissolving solvent were added and the solution sonicated for 5 min.

For tablets, a single tablet was placed into a stoppered flask, 0.7 ml of internal standard and 6.3 ml of dissolving solvent were added. The flask was sonicated with occasional shaking until a fine even dispersion was obtained then an aliquot of this solution was centrifuged at $1500 \times g$ for 10 min to obtain a clear supernatant.

All solutions were stored at 2–8°C prior to injection.

2.6. Standard solution

Into a 1000 ml volumetric flask, 0.2 ml of methanesulphonic acid was accurately transferred. The methanesulphonic acid was dispersed in and diluted to volume with dissolving solvent, then 5 ml of this solution and 25 ml of internal standard solution were transferred into a 250 ml flask and diluted to volume with dissolving solvent.

2.7. Procedure

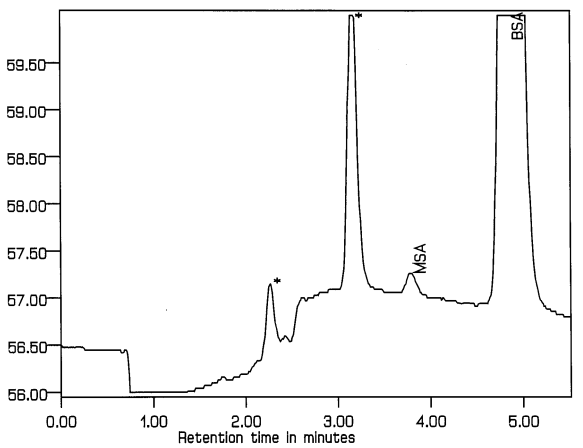
Eluent and suppressant were pumped through the column and suppressor until a stable baseline was obtained. Injections of standard solutions were made followed by the sample solutions. The analysis was concluded with bracketing standard injections. For the standard and sample solutions the methanesulphonic acid peak areas were all divided by the internal standard peak areas. By comparison of the corrected peak areas of the samples and bracketing standards, the percentage of methanesulphonic acid present was calculated.

3. Results and discussion

3.1. Validation

Commercial scale batches of drug substance and pilot scale batches of tablets for use in formal stability studies were used in the validation of this procedure. Validation was carried out specifically for the determination of methanesulphonic acid in busulfan and in busulfan (Myleran®) 2 mg tablets.

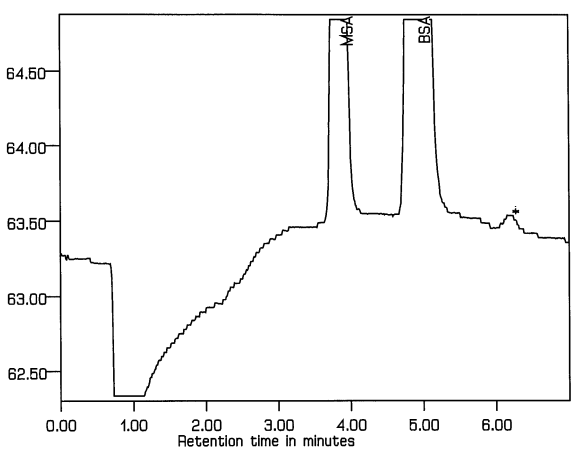
The following experiments, which validate the method for drug substance, are equally applicable to the assay of reformulated busulfan (Myleran®) 2 mg tablets. Assuming no interference from excipients, the method should also be applicable to other busulfan tablet formulations.



* Peaks due to components of the formulation

MSA = Methanesulphonic Acid
BSA = Butanesulphonic Acid

Fig. 1. Demonstration of the sensitivity of the procedure for methanesulphonic acid detection at 0.05% w/w.



* Peaks attributable to dissolving solvent

MSA = Methanesulphonic Acid
BSA = Butanesulphonic Acid

Fig. 2. Typical chromatogram obtained for standard solution.

3.2. Linearity of the method

The linearity of the method over the concentration range 0.295–14.73 μg/ml of methanesulphonic acid (equivalent to 0.1–4.9% of the nominal busulfan concentration) was investigated by injecting a series of solutions containing methanesulphonic acid and internal standard, at the appropriate concentrations, onto the chromatographic system. The peak area ratio of methanesulphonic acid to butanesulphonic acid was then determined. Two injections were made of each solution, and duplicate readings obtained, therefore the mean of the two results was used in the analysis rather than using a lack of fit test. Three statistical tools were used to evaluate the linearity of the method. A weighted analysis, a transformation of peak areas and methanesulphonic acid concentrations using logarithms and linear regression were all performed on the data.

To account for unequal variances across the range of concentrations a weighted analysis was performed on the data, by weighting the peak area ratio by 1/variance for the relevant concentration level. A model was then fitted to the weighted data to ascertain whether or not a linear relationship existed between peak area and concentration. The relative standard deviations for the peak area ratios of duplicate injections were quite variable once the size of the mean had been accounted for and this affected the model and the residuals. Using the weighted analysis seemed to indicate that there was some curvature in the data.

A model was fitted to the log–log transformation of peak area ratio and concentration data and a P-value calculated to ascertain whether there was a linear relationship between the two. Using a 5% significant level, there was evidence for the hypothesis that a linear relationship existed ($P < 0.0001$). Residuals plotted for the modelled data were randomly scattered around the line zero, showing that the model fitted to the data was optimal. There was some evidence of curvature but fitting the quadratic model showed that the additional term was not significant therefore it was concluded that the relationship between peak area ratio and acid concentration was linear.

Table 1
Accuracy of the analytical procedure

Methanesulphonic acid content (as % of nominal busulfan content)	Methanesulphonic acid recovered (%) individual values			Mean (%)
0.05	98.6	96.6	99.8	98.3
0.10	103.2	104.2	98.4	101.9
0.3	102.8	99.9	96.2	99.6
0.5	99.9	98.9	96.8	98.5
1.0	100.2	102.1	98.5	100.2
5.0	111.0	111.7	111.6	111.4
			Overall mean	101.7
			RSD	4.9%

Using linear regression, a linear response for peak area ratio versus methanesulphonic acid concentration was also obtained: $y = 0.06580x - 0.0066$ (correlation coefficient = 0.9991).

Based on the statistical evaluations performed, the linearity of detector response was therefore found to be acceptable.

The limit of detection and limit of quantitation for methanesulphonic acid were both determined by the defined method. The limit of detection, was determined as the lowest amount of methanesulphonic acid which could be detected but not necessarily quantitated using the chromatographic procedure. This was determined to be 0.02% w/w with respect to the nominal busulfan content. The limit of quantitation was determined as the lowest amount of methanesulphonic acid which could be quantitatively determined with suitable accuracy and precision using the chromatographic procedure. This was determined to be 0.05% w/w with respect to the nominal busulfan content.

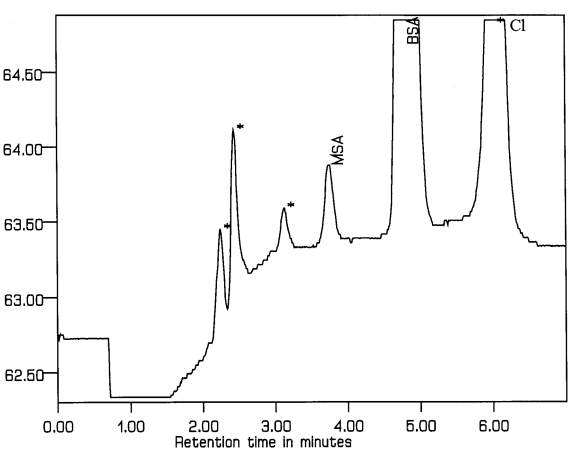
The chromatogram given in Fig. 1 shows that the sensitivity of the ion chromatography procedure was adequate for the quantitation of methanesulphonic acid at a level of 0.05% w/w.

3.3. Specificity

To demonstrate the specificity of the chromatographic system and to demonstrate that the method was stability indicating, solutions of other potential degradation impurities of busulfan, namely butan-1,4-diol and tetrahydrofuran, were chromatographed using the defined conditions. A placebo formulation (omitting only the busulfan) unstressed and following storage for 17 days at 50°C, was also chromatographed according to the defined method. Comparison of the chromatograms with that obtained for a standard solution showed no evidence of any interference with the peaks due to methanesulphonic acid and butanesulphonic acid (see Fig. 2).

3.4. Accuracy

The accuracy of the method was determined by recovery experiments. In triplicate experiments,



* Peaks attributable to excipients in the tablet formulation

MSA = Methanesulphonic Acid
BSA = Butanesulphonic Acid
Cl = Chloride

Fig. 3. Typical chromatogram obtained for tablet sample solution.

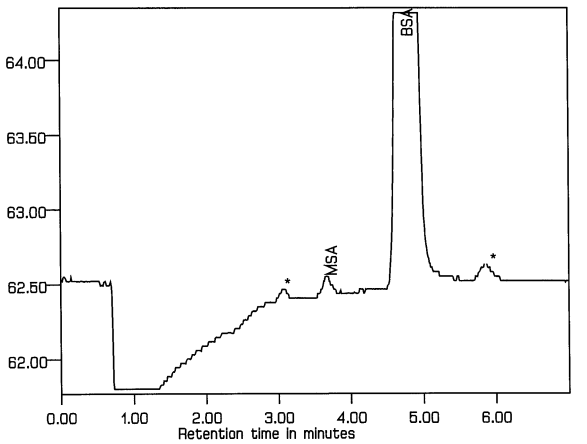


Fig. 4. Typical chromatogram obtained for drug substance sample solution.

known quantities of methanesulphonic acid were added to a placebo formulation over the range 0.05–5% of the nominal busulfan sample concentration employed during the analytical procedure. These samples were then tested by the method. Data indicate an overestimation of methanesulphonic acid content at the 5% level, all other values being very close to nominal values. An additional experiment to assess the accuracy of the method for a 2% level of methanesulphonic acid in busulfan drug substance gave results very close to nominal values. The data shown in Table 1 demonstrate the accuracy of the analytical procedure to be acceptable, since the levels of methanesulphonic acid in drug substance and tablet batches are normally at or below the 2% level (see Fig. 3 and Fig. 4).

3.5. Precision

The repeatability, intermediate precision and reproducibility were assessed for the analytical method. The repeatability of measurement for methanesulphonic acid was determined by making ten replicate injections of a single standard solution, onto the chromatographic system. A relative standard deviation (RSD) of 0.3% was obtained. Additionally, the repeatability of preparation for standard solutions was determined by preparing ten standard solutions containing methanesulphonic acid, injecting each singly and calculating the mean response factor. An RSD of 0.5% was obtained. The repeatability of measurement and repeatability of preparation for standard solutions were found to be satisfactory.

The intermediate precision of the analytical procedure for tablets and drug substance was measured by determining the methanesulphonic acid content of batches of tablets on different days by different analysts. The results are given in Table 2 and Table 3, respectively.

Analysis of variance (ANOVA) was performed on the drug substance and tablet data, and the

Table 2
Intermediate precision of the analytical procedure for tablets

	Methanesulphonic acid content (as % of nominal busulfan content)					
	Batch 1		Batch 2		Batch 3	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
	0.29	0.27	0.10	0.12	0.23	0.15
	0.30	0.29	0.09	0.12	0.22	0.17
	0.28	0.29	0.08	0.16	0.24	–
	0.24	0.29	0.08	0.14	0.27	–
	0.27	0.32	0.07	0.15	0.26	–
Mean	0.28	0.29	0.08	0.14	0.24	0.16
Overall mean	0.28		0.11		0.20	

Table 3
Intermediate precision for the analytical procedure for drug substance

	Methanesulphonic acid content (as % of nominal busulfan content)					
	Batch 1		Batch 2		Batch 3	
	Analyst 1	Analyst 2	Analyst 1	Analyst 2	Analyst 1	Analyst 2
Individual values	< 0.05	<0.05	< 0.05	<0.05	< 0.05	<0.05
	< 0.05	<0.05	< 0.05	<0.05	< 0.05	<0.05
	< 0.05	<0.05	< 0.05	<0.05	< 0.05	<0.05
	< 0.05	<0.05	< 0.05	<0.05	< 0.05	0.06
	< 0.05	<0.05	< 0.05	<0.05	< 0.05	0.05
Mean	< 0.05	<0.05	< 0.05	<0.05	< 0.05	<0.05
Overall mean	<0.05		<0.05		<0.05	

Table 4
ANOVA results for intermediate precision of the analytical procedure for busulfan drug substance

Source	Sum of squares	Mean sum of squares	Number of degrees of freedom	F ratio	Prob > F
Batch	0.00054	0.00027	2	10.7351	0.0852
Analyst	1.67×10^{-7}	1.67×10^{-7}	1	0.0066	0.9426

Table 5
ANOVA results for intermediate precision of the analytical procedure for busulfan tablets

Source	Sum of squares	Mean sum of squares	Number of degrees of freedom	F ratio	Prob > F
Batch	0.03064	0.01532	2	5.9255	0.1444
Analyst	8.17×10^{-6}	8.17×10^{-6}	1	0.0032	0.9603

results in Tables 4 and 5, respectively, conclude analyst to analyst variability to be minimal.

The intermediate precision of the method was therefore acceptable, for both drug substance and busulfan (Myleran®) 2 mg tablets.

The reproducibility of the analytical procedure (site) was determined by analysing the methanesulphonic acid content of three batches of drug substance, by two different sites. The results are given in Table 6. ANOVA results for reproducibility are shown in Table 7.

At site one the samples were prepared using automated means, whilst site two samples were prepared manually. Given the unstable nature of busulfan to hydrolyse when in aqueous solution, there is some variation in results between sites, due purely to the differences in sample preparation time prior to injection, for automated versus manual preparation. In practicality, the differences between laboratory results are negligible, due to the differences in sample preparation time and rate of hydrolysis. The reproducibility of the analytical procedure between sites was therefore considered to be acceptable.

3.6. Stability of solutions

Busulfan is known to hydrolyse in aqueous solution. To assess the rate of hydrolysis of the samples, a solution of busulfan was prepared in

Table 6
Demonstration of the reproducibility of the analytical procedure

	Mean methanesulphonic acid content (as % of nominal busulfan content)	
	Site 1	Site 2
Batch 1	0.02	0.08
Batch 2	0.02	0.08
Batch 3	0.04	0.07

duplicate, and the methanesulphonic acid content determined using the defined analytical procedure. After storage for 43 h at ambient temperature (20–30°C) and refrigerated (2–8°C) the methanesulphonic acid content was determined again. The data show a rate of hydrolysis of 0.02%/h at 2–8°C and 0.11%/h at 20–30°C, assuming first order reaction kinetics for the degradation of busulfan to form methanesulphonic acid. It was concluded that sample solutions should be stored at 2–8°C and injected within one hour of initiating sample preparation.

The stability of the standard solution was determined by preparing a solution of methanesulphonic acid working standard in duplicate, and determining the methanesulphonic acid content using the defined analytical procedure. The data showed no significant change in methanesulphonic acid content after 14 days storage at ambient temperature (20–30°C) in a sealed container.

To assess the stability of the reagents, the resolution between methanesulphonic acid and butanesulphonic acid was determined by the method of the British pharmacopoeia, using the defined analytical procedure. The retention time of methanesulphonic acid was also recorded. The initial resolution and retention time were 4.12 and 3.85 min, respectively. After storage of the mobile phase for 14 days at ambient temperature (20–30°C) in a sealed container, the resolution and retention times were redetermined. These were found to be 4.14 and 3.84 min, respectively. These data show no significant change in the resolution between methanesulphonic acid and butanesulphonic acid, nor in the retention time of methanesulphonic acid, after storage for up to 14 days at ambient temperature.

3.7. Robustness

The robustness of the method was tested, using a 12 run experimental design based on 1/2 2^4 fractional factorial. A placebo tablet solution was prepared spiked to contain 2% w/w of methanesulphonic acid and an internal standard level the same as for a busulfan 2 mg tablet preparation. Mobile phase concentration, injection volume, temperature and flow rate, were varied around the method values and the methanesulphonic acid content determined by the defined procedure. The resolution between methanesulphonic acid and butanesulphonic acid was also measured. The conclusions derived from statistical analysis of the results shown in Table 8 demonstrated that although temperature was a statistically significant factor, affecting the resolution of methanesulphonic acid from butanesulphonic acid, the range of responses produced was considered analytically robust.

4. Conclusions

A robust and sensitive ion chromatography procedure has been developed to allow rapid and

Table 7
ANOVA results for reproducibility of the analytical procedure for drug substance

Source	Sum of squares	Mean sum of squares	Number of degrees of freedom	F ratio	Prob $> F$
Batch	8.33×10^{-6}	4.17×10^{-6}	2	0.0400	0.9615
Analyst	0.004	0.004	1	38.4400	0.0250

Table 8
Factors investigated to demonstrate robustness of the analytical procedure

Run	Factors investigated				Responses monitored	
	Temperature (°C)	Eluent concentration (% w/w)	Injection volume (μl)	Eluent flow rate (ml/min)	Resolution	Methanesulphonic acid content (% w/w)
1	35	0.0154	60	2.2	4.24	3.868
2	30	0.0140	50	2.0	4.15	3.857
3	30	0.0140	50	2.0	4.15	3.849
4	35	0.0154	40	1.8	4.48	3.936
5	25	0.0154	40	2.2	4.01	3.866
6	30	0.0140	50	2.0	4.17	3.863
7	25	0.0126	40	1.8	3.89	3.753
8	25	0.0154	60	1.8	3.72	3.775
9	35	0.0126	40	2.2	4.46	3.756
10	30	0.0140	50	2.0	4.14	3.846
11	35	0.0126	60	1.8	4.53	3.858
12	25	0.0126	60	2.2	3.81	3.829

automatable determination of the methanesulphonic acid content of busulfan samples.

The current pharmacopoeial tests for busulfan drug substance and tablets include a test for acidity, where an amount of busulfan has aqueous sodium hydroxide solution added to it in the presence of an indicator. Busulfan tablets contain magnesium stearate, which is derived from stearic and palmitic acids, which would also be neutralised by hydroxide, as well as any methanesulphonic acid present; therefore this is a nonspecific test. By using ion chromatography, the method shows specificity to methanesulphonic acid and therefore is more stability indicating than current procedures. For the current impurity method a quantity of powdered tablet equivalent to 20 mg of busulfan is passed through the assay procedure. The new method can be used on individual 2 mg tablets or small quantities, typically 5 mg, of drug substance. Due to the cytotoxic nature of busulfan, the minimisation of handling of this substance can be achieved by the reduction in volumes and lower quantities of material. Therefore this method offers improved sensitivity, over the existing method in the analysis of smaller samples.

The method has been validated for a 2 mg tablet formulation.

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