Determination of methanesulfonic acid in busulfan by ion chromatography

Authors: Manali Aggrawal and Jeffrey Rohrer
Thermo Fisher Scientific, Sunnyvale, CA, USA

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Goals

1. To evaluate the limit test for MSA in the proposed USP Busulfan monograph

2. To evaluate the same limit test with modifications

Introduction

Busulfan (1,4-butanediol dimethanesulfonate) is a bifunctional alkylating agent used in the treatment of chronic myelogenous leukemia.[1,2] The drug is administered either as a tablet formulation or injectable medication, typically at a dosage level of 2 mg per tablet and 6 mg/mL injectable solution. Busulfan undergoes a hydrolysis in aqueous media and forms methanesulphonic acid (MSA) and tetrahydrofuran.[3] The presence of MSA in busulfan solution indicates the instability of the drug substance. Therefore, monitoring MSA levels can give an accurate indication of the busulfan stability. In addition, MSA may be toxic above certain concentrations. Thus, a selective procedure is required for determining MSA in busulfan drug substance. The current United States Pharmacopeia–National Formulary (USP–NF) monograph for busulfan does not have a test for MSA impurity.[4] As part of the United States Pharmacopeia's (USP) monograph modernization efforts, it is proposed in the USP's Pharmacopeial Forum (USP-PF) to add the test for "Limit of Methanesulfonic Acid" in the Busulfan monograph[5]. This test uses an ion chromatography (IC) method based on analyses performed with a Thermo Scientific™ Dionex™ IonPac™ AG11-HC guard column with L61 packing and Thermo Scientific Dionex IonPac AS11-HC analytical column with L81 packing, respectively.

In this application note, we evaluated the proposed USP Busulfan monograph method for the “Limit of Methanesulfonic Acid”. MSA was separated on a Dionex IonPac AS11-HC column set followed by suppressed conductivity detection. The method was further evaluated with a slight modification to the sample preparation method. The busulfan sample was prepared in cold 30:70 acetonitrile:water (30% MeCN) instead of water. We also set the autosampler temperature set to 8 °C instead of 25 °C. Key performance parameters were evaluated including separation, system suitability, linearity, limit of detection, and precision. Three busulfan samples were analyzed. The percentage of MSA results were compared with the acceptance criterium in the proposed USP monograph.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000 HPIC system including:
  - Dionex ICS-6000 DP Pump module
  - Dionex ICS-6000 EG Eluent Generator module with high-pressure degasser module
  - Dionex ICS-6000 Low Temperature DC Detector/ Chromatography module with two injection valves
  - CD Conductivity Detector
  - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control (P/N 074926), sample syringe, 250 µL (P/N 074306) and buffer line, 1.2 mL (P/N 074989)
- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 Continuously Regenerated Anion Trap Column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm, P/N 088667)
- Thermo Scientific™ Dionex™ IC PEEK Viper Fitting Tubing Assembly Kit (2 mm, P/N 302965)
- Dionex AS-AP Autosampler Vials 10 mL (P/N 074228)

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistance or better
- Thermo Scientific™ Dionex™ Combined Seven Anion Standard II (P/N 057590)
- Methanesulfonic Acid 99.0+%, TCI America™ (P/N M205925G)
- Thermo Scientific™ HyperSep™ C18 cartridges (P/N 03-251-156)
- Busulfan, European Pharmacopoeia (EP) Reference Standard, Sigma-Aldrich Fine Chemicals Biosciences (P/N B1170000)
- Busulfan, analytical standard (AS), for drug analysis, MilliporeSigma (P/N B2635-10G)
- Busulfan, British Pharmacopoeia (BP) Reference Standard, Sigma-Aldrich Fine Chemicals Biosciences (P/N BP403)

Standard stock solution

0.03 mg/mL methanesulfonic acid in acetonitrile

Accurately weigh 6 mg of MSA standard in a 200 mL plastic volumetric flask and add acetonitrile up to the mark to make 0.03 mg/mL stock standard. Sonicate and mix well for one minute.

Standard solution

1.5 µg/mL of methanesulfonic acid in water, from standard stock solution

Dilute the stock standard 20× to make 1.5 µg/mL of MSA in DI water*. Use a freshly conditioned 500 mg solid phase extraction cartridge with C18 packing to perform the extraction. The cartridge is conditioned as follows. Sequentially rinse the cartridge with 6 mL of acetonitrile, 6 mL of water, and 1.5 mL of standard solution. Load 1.5 mL of standard solution on the cartridge, perform the extraction at a drop per second as soon as possible after preparation, and collect the solution for analysis.

*For the modified method, use cold 30% MeCN solution instead of DI water.

Calibration standards

Dilute volumes of the 0.03 mg/mL stock solution listed below to 10 mL with 30% MeCN to prepare the following calibration standards of MSA: 0.1, 0.25, 0.5, 1, 2.5, and 5 µg/mL.

Calibration standard (µg/mL)	Stock volume added (µL)
0.10	33.3
0.25	83.3
0.50	167
1.00	333
2.50	833
5.00	1667

Sample stock solution

20 mg/mL of busulfan in acetonitrile

Accurately weigh 50 mg of the busulfan sample in a 2.5 mL plastic volumetric flask and add acetonitrile up to the mark to make a 20 mg/mL stock sample. Sonicate and mix well for one minute.

Sample solution

1 mg/mL of busulfan in water, from sample stock solution

Dilute the stock standard 20× to make 1 mg/mL busulfan in DI water*. Then perform the extraction as explained in the above section for the standard solution.

*For the modified method, use cold 30% MeCN solution instead of DI water.

Spike recovery experiment for the modified method

Unspiked sample: Accurately measure 500 µL of the 20 mg/mL busulfan sample in a 10 mL glass volumetric flask and add cold 30% MeCN up to the mark to make a 1 mg/mL solution.

Spiked 1 sample: To prepare a 0.1 µg/mL spiked sample, measure 500 µL of the 20 mg/mL busulfan sample in a 10 mL volumetric flask and add 33.3 µL of 0.03 mg/mL MSA, then bring to volume with cold 30% MeCN.

Spiked 2 sample: To prepare a 0.2 µg/mL spiked sample, measure 500 µL of the 20 mg/mL busulfan sample in a 10 mL volumetric flask and add 66.6 µL of 0.03 mg/mL MSA, then bring to volume with cold 30% MeCN.

Table 1. Chromatography conditions (method in the proposed USP monograph)

Parameter	Value
System	Dionex ICS-6000
Columns	Dionex IonPac AS11-HC, Analytical, 2 × 250 mm (P/N 052961) Dionex IonPac AG11-HC, Guard, 2 × 50 mm (P/N 052963)
Eluent source	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 600 trap column
Analytical gradient	Time [min] KOH (mM) 0 1 2 1 13 15 20 71 20 1 28 1
Flow rate	0.3 mL/min
Column temp.	35 °C
Autosampler temp.	25 °C
Injection vol.	10 µL
Detection	Suppressed conductivity
Suppressor	Dionex ADRS 600 Suppressor (2 mm) (P/N 088667), recycle mode, 4.0 V, constant voltage mode
Run time	28 min

Table 2. Proposed USP method and modifications

	Proposed USP method	Modified method
Autosampler temperature	25 °C	8 °C
Preparation of 1 mg/mL busulfan	DI water	Cold 30% MeCN
Preparation of 1.5 µg/mL MSA standard	DI water	Cold 30% MeCN

Results and discussion

The proposed USP Busulfan monograph describes a Dionex IonPac AG11-HC guard column with L61 packing and a Dionex IonPac AS11-HC analytical column with L81 packing for the separation of MSA. The Dionex IonPac AS11-HC column is a high capacity hydroxide selective, strong anion exchange column consisting of ethylvinylbenzene cross-linked with 55% divinylbenzene and latexed with smaller particles containing alkanol quaternary ammonium ions.[6] Figure 1 displays the chromatographic profile of a 1.5 µg/mL MSA standard solution using the gradient eluent condition as described in proposed USP monograph method (Table 1). Eluent was generated electrolytically using a Dionex EGC 500 KOH

cartridge. The MSA peak is well resolved from contaminant anions present in the MSA standard solution. These contaminant anions are: acetate, chloride, and sulfate. The acetate is likely from the water and we believe chloride and sulfate leached from the C18 cartridge used for extraction. The retention time (RT) for MSA is ~9.44 min. According to the proposed monograph, MSA elutes at approximately 9.5 min.[5] The separation is followed by suppressed conductivity detection.

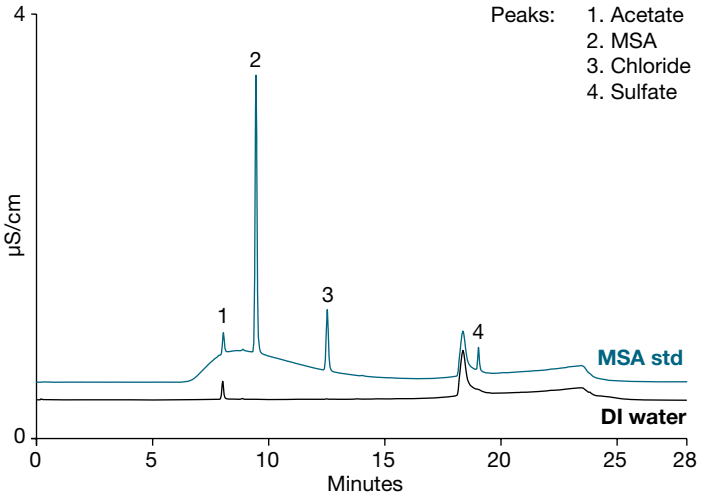

Figure 1. Chromatograms of DI water and 1.5 µg/mL methanesulfonic acid (MSA) standard prepared in DI water (5% signal offset is applied)

Stability of busulfan

As per the USP monograph, a stock sample solution of busulfan is prepared in acetonitrile and then diluted with DI water to prepare the working solution. Busulfan in aqueous solution is extremely unstable and known to hydrolyze to MSA. To assess the rate of hydrolysis of the busulfan, a solution of busulfan was prepared in two solvents and each at two temperatures; water (room temperature), cold water (~2–8 °C), 30% MeCN (room temperature), and cold 30% MeCN (~2–8 °C). The hydrolysis of busulfan was assessed by injecting the prepared solutions into the IC system at times, t = 0, 1, 4, and 15 h and determining the peak area response of MSA. We found the stability of busulfan is as follows: cold 30% MeCN > cold water > 30% MeCN > water (Figure 2). It is evident that busulfan solution is more stable in cold 30% MeCN with storage at 8 °C. Although it is relatively stable, it still degrades to MSA but at a slower rate than that in water. We decided that sample solutions should be prepared in cold 30% MeCN, stored at 2–8°C, and injected within 1–2 h of initiating sample preparation. The stability of the MSA standard solution was also assessed by preparing a solution of MSA working standard in both water and 30% MeCN and determining the MSA content using the defined analytical procedure. The data showed no significant change in MSA content after 14 days storage at ambient temperature (20–30 °C) in a sealed container.

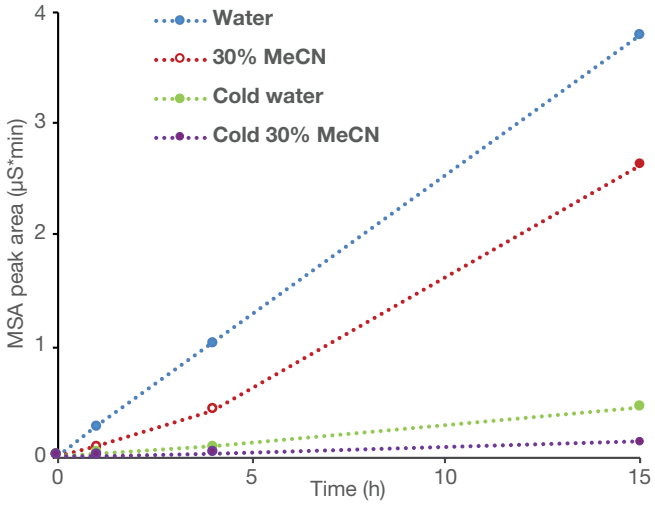

Figure 2. Plot of MSA peak area vs. time

Figure 3 displays the chromatograms of MSA standard prepared in DI water and 30% MeCN. A bump in the chromatogram's baseline between 5 to 12 min is observed in the chromatogram of MSA prepared in 30% MeCN. The MSA peak elutes on the slope of this bump at the same RT as in DI water. The peak response of MSA prepared in 30% MeCN is approximately 1.3 times lower than that prepared in DI water. Therefore, it is important that standards and samples be prepared in the same manner.

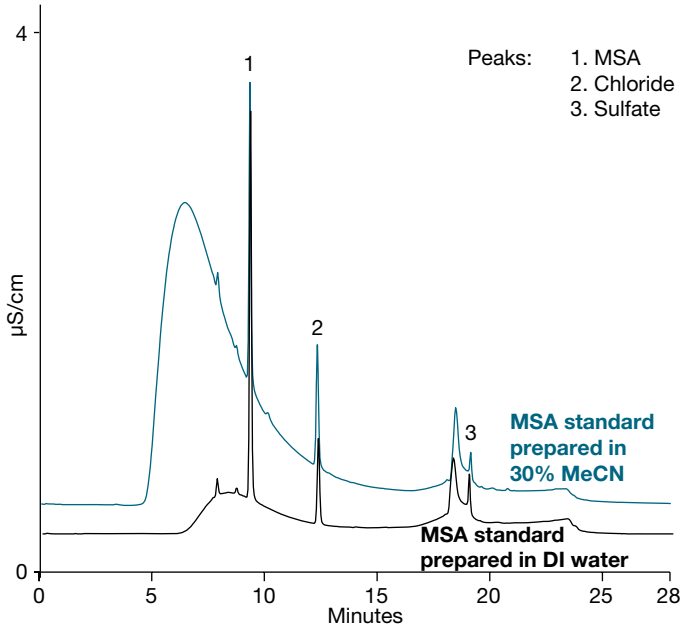

Figure 3. Chromatograms of 1.5 µg/mL MSA standard prepared in DI water and 30% cold MeCN (5% signal offset is applied)

System suitability

In the proposed USP monograph for busulfan, two system suitability requirements are specified. These requirements are that the MSA peak tailing factor for a 1.5 µg/mL MSA standard solution is not more than (NMT) 2.0 and that the relative standard deviation (RSD) for replicate injections of 1.5 µg/mL MSA standard solution is NMT 5%. The RSD of the retention time, peak area, and peak height were determined from six replicate injections of the MSA standard solution in both water and 30% MeCN. Table 3 shows that the USP requirements for tailing factor and repeatability are met with both methods.

Table 3. System suitability

	Tailing factor	Peak area RSD (n=9)	Retention time RSD (n=9)	Peak height RSD (n=9)
Proposed USP	1.11	2.47	0.05	1.08
Modified	1.13	2.61	0.07	2.48

Linearity and limits of detection (LOD) and quantitation (LOQ)

Method linearity was evaluated by constructing calibration curves using six concentrations of MSA standard ranging from 0.1 to 5 µg/mL. The calibration plot of peak area versus concentration was fit using linear regression yielding a coefficient of determination (r^2) greater than 0.999.

To determine the LOD and LOQ, the baseline noise (N) was first determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute, but close to the MSA peak. The signal (S) was determined from the average peak height of three injections of 5 µg/L and 10 µg/L MSA standards for the proposed USP and modified methods, respectively. As mentioned earlier, the peak response of MSA prepared in 30% MeCN is lower than that prepared in DI water. Thus, for the modified method we doubled the concentration of MSA to determine the LOD and LOQ. The LOD and LOQ were determined by 3 times and 10 times the S/N, respectively. Table 4 lists and compares the LOD and LOQ values of MSA prepared by the proposed USP method and the modified method. Although, the LOD and LOQ values determined by the modified method are 2.5 times higher than those determined by the proposed USP method, the values are low enough to meet the limit of MSA test requirements.

Table 4. Method calibration, LOD, and LOQ data for MSA

Method	Linearity (r^2)	LOD (µg/L)	LOQ (µg/L)
Proposed USP	0.9999	1.25	4.16
Modified	0.9998	3.45	11.52

Robustness study

Following the guidelines of the USP Physical Tests, <621> Chromatography[7], the robustness of this method was evaluated by examining retention time (RT) and peak asymmetry, after imposing small variations (±10%) in procedural parameters (e.g., flow rate, eluent concentration, column temperature). 1.5 µg/mL MSA standard solution was injected in triplicate for each condition for three consecutive days. The same procedure was applied to another column set from a different lot. Figure 4 displays chromatograms of a 1.5 µg/mL MSA standard on two columns. The RTs of the MSA peak on two columns were found to differ by ~2.13%; i.e., 9.44 min on Column 1 and 9.24 min on Column 2.

The variations tested were:

- Flow rate at 0.27 mL/min, 0.30 mL/min, and 0.33 mL/min
- Column temperature at 31.5 °C, 35 °C, and 38.5 °C
- Eluent concentration:

	KOH (mM)		
Time (min)	Eluent -10%	Eluent	Eluent +10%
0–2	0.9	1	1.1
13	13.5	15	16.5
20	63.9	71	78.1
20	0.9	1	1.1
28	0.9	1	1.1

Tables 5 and 6 summarize the results of the method robustness study for both the proposed USP and modified methods, respectively. These results indicate that the methods are robust and suitable for MSA determinations.

Table 5. Robustness of the proposed USP method for MSA determination performed using a 1.5 µg/mL MSA standard (n=9)

Parameter		Column 1				Column 2			
		RT		Asymmetry		RT		Asymmetry	
		Avg (n=9)	% diff.	Avg (n=9)	% diff.	Avg (n=9)	% diff.	Avg (n=9)	% diff.
	No change	9.444	0.00	1.10	0.00	9.243	0.00	1.05	0.00
Eluent	Eluent +10%	9.183	2.76	1.11	-0.91	8.970	2.95	1.06	-0.95
	Eluent -10%	9.741	-3.14	1.09	0.91	9.525	-3.05	1.04	0.95
Flow rate	0.27 mL/min	10.118	-7.14	1.09	0.91	9.886	-6.96	1.05	0.00
	0.33 mL/min	8.879	5.98	1.09	0.91	8.684	6.05	1.05	0.00
Column temp.	27 °C	9.336	1.14	1.09	0.91	9.127	1.26	1.06	-0.95
	33 °C	9.552	-1.14	1.11	-0.91	9.331	-0.95	1.05	0.00

Table 6. Robustness of the modified method for MSA determination performed using a 1.5 µg/mL MSA standard (n=9)

Parameter		Column 1				Column 2			
		RT		Asymmetry		RT		Asymmetry	
		Avg (n=9)	% diff.	Avg (n=9)	% diff.	Avg (n=9)	% diff.	Avg (n=9)	% diff.
	No change	9.447	0.00	1.13	0.00	9.233	0.00	1.08	0.00
Eluent	Eluent +10%	9.183	2.79	1.13	0.00	8.969	2.86	1.10	-1.85
	Eluent -10%	9.738	-3.08	1.11	1.77	9.519	-3.10	1.08	0.00
Flow rate	0.27 mL/min	10.113	-7.05	1.12	0.88	9.884	-7.05	1.09	-0.93
	0.33 mL/min	8.881	5.99	1.09	3.54	8.686	5.92	1.07	0.93
Column temp.	27 °C	9.338	1.15	1.12	0.88	9.137	1.04	1.09	-0.93
	33 °C	9.552	-1.11	1.10	2.65	9.328	-1.03	1.09	-0.93

Sample analysis

Three commercial busulfan samples (Table 7) were tested using both methods. Figures 5 and 6 display the chromatograms of Samples A, B, and C using the proposed USP and modified methods respectively.

Table 7. Limit of MSA

Sample	Busulfan sample	Method	r_u	r_s	C_s	C_u	Result (NMT 0.15%)
A	EP Reference std. (Product code B1170000)	USP proposed	0.085	0.297	0.0015	1	0.043
		Modified	0.015	0.246	0.0015	1	0.009
B	BP Reference std. (Product code BP403)	USP proposed	0.308	0.297	0.0015	1	0.156
		Modified	0.055	0.246	0.0015	1	0.034
C	AS (Product code B2635)	USP proposed	3.75	0.297	0.0015	1	1.89
		Modified	3.11	0.246	0.0015	1	1.90

r_u = peak response of methanesulfonic acid from the Sample solution
r_s = peak response of methanesulfonic acid from the Standard solution
C_s = concentration of USP Methanesulfonic Acid RS in the Standard solution (mg/mL)
C_u = concentration of Busulfan in the Sample solution (mg/mL)

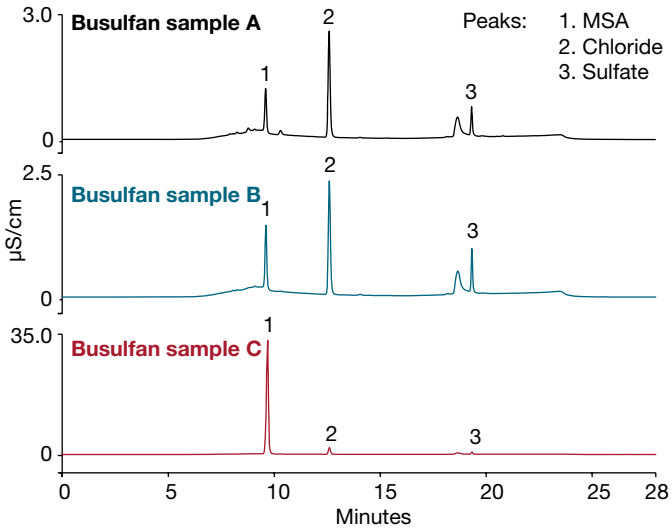

Figure 5. Chromatograms of busulfan samples A, B, and C, prepared in DI water

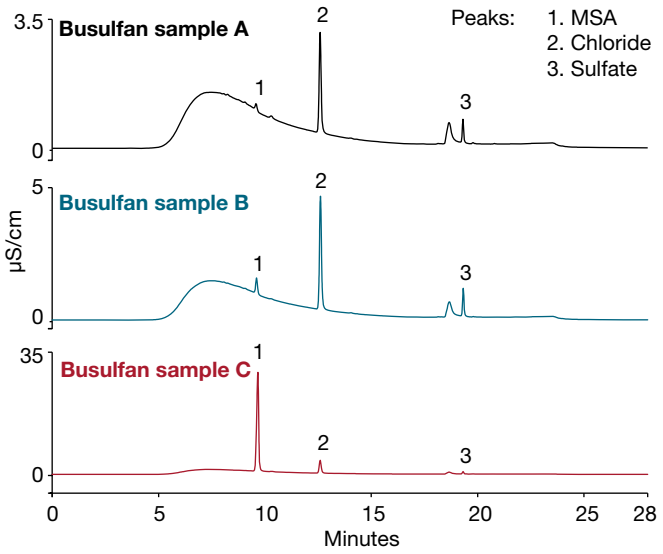

Figure 6. Chromatograms of busulfan samples A, B, and C, prepared in 30% cold MeCN

It is evident from the response in both figures that Sample C contains significantly more MSA impurity than Samples A and B. The percentage of MSA in all three samples was calculated as specified in the proposed monograph:

$$\text{Percentage} = (r_U / r_S) \times (C_S / C_U) \times 100$$

Where:

r_U = peak response of methanesulfonic acid from the sample solution

r_S = peak response of methanesulfonic acid from the standard solution

C_S = concentration of USP methanesulfonic acid RS in the standard solution (mg/mL)

C_U = concentration of busulfan in the sample solution (mg/mL)

The USP acceptance criterium for the MSA content in busulfan is that the product should contain less than 0.15% MSA. Using the modified method, both Samples A and B passed the limit test, whereas Sample C failed the test with a significantly high amount of MSA (Table 7).

Using the proposed USP method, only Sample A passed the limit test whereas Samples B and C failed the test. This is due to the fact that busulfan degrades to MSA at a faster rate in DI water and at 25 °C temperature (proposed USP method conditions). Thus the sample should be injected/analyzed as soon as it is prepared. To determine the rate of degradation of busulfan to MSA, Sample B (Busulfan BP) was prepared using both the proposed and modified USP methods and injected at different times. Table 8 shows the results of this experiment for Sample B. Sample B passed the limit test under both the proposed and modified USP methods when it was injected within 10–12 min of sample preparation. Sample B failed the test using the proposed USP method when injected ≥40 min after the preparation, whereas it passed the limit test using the modified USP method <30 h after the sample preparation. Although the busulfan sample passes the limit of the MSA test using the modified USP method at longer wait times, it is best to analyze the sample within 1–2 h of sample preparation for accurate results.

Table 8. Limit of MSA (Busulfan, BP)

Busulfan, BP Reference std., (Product code BP403)							
Method	Time gap between sample prep. and injection	r_u	r_s	C_s	C_u	Result (NMT 0.15%)	
USP proposed	~10-12 min.	0.111	0.297	0.0015	1	0.056	Passed
	~40-42 min.	0.308	0.297	0.0015	1	0.156	Failed
Modified	~10-12 min.	0.050	0.246	0.0015	1	0.030	Passed
	~40-42 min.	0.055	0.246	0.0015	1	0.034	Passed
	~6 h	0.082	0.246	0.0015	1	0.05	Passed
	~12 h	0.115	0.246	0.0015	1	0.07	Passed
	~18 h	0.155	0.246	0.0015	1	0.10	Passed
	~30 h	0.265	0.246	0.0015	1	0.162	Failed

Sample recovery

Method accuracy was evaluated by measuring recoveries of MSA standard spiked at two concentrations. The unspiked and spiked samples were prepared in 30% cold MeCN (modified method). The recovery percentages were calculated according to formula given below:

$$Recovery\ \% = \frac{C\ spiked\ sample - C\ unspiked\ sample}{C\ analyte\ added} * 100$$

Figure 7 shows the representative chromatograms of unspiked Sample A and Sample A spiked with 0.1 µg/mL and 0.2 µg/mL MSA standard. The recovery of two spiked levels in both the samples was in the range of 90 to 110% (Table 9).

We also evaluated the accuracy of the proposed USP method by preparing unspiked and spiked busulfan sample in DI water. The MSA recovery percentages obtained were very high >500%. This is due to the rapid degradation of busulfan sample to MSA in aqueous solution at room temperature.

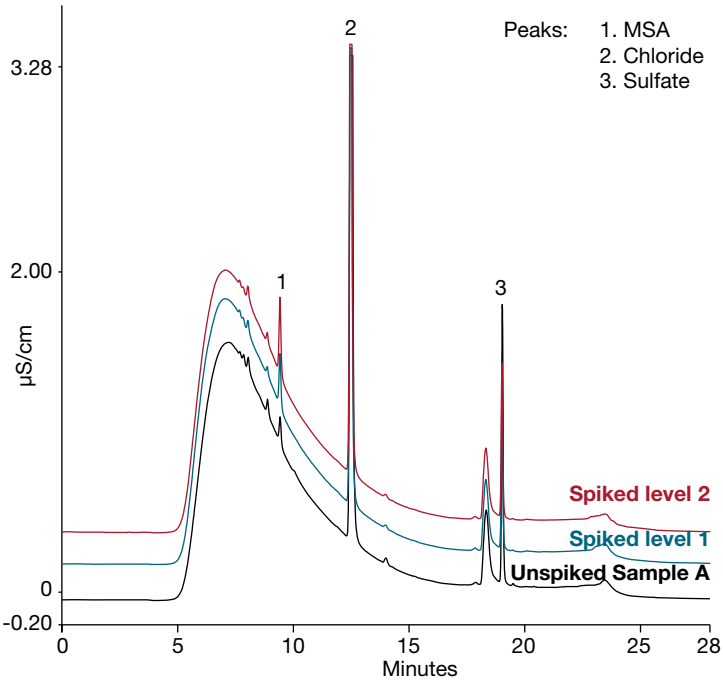

Figure 7. Chromatograms of Sample A and Sample A spiked with 0.1 and 0.2 µg/mL MSA standard, prepared in 30% cold MeCN (5% signal offset is applied)

Table 9. MSA recovery study (n=3)

Busulfan sample	Base amount (µg/mL)	Spiked amount (µg/mL)	Measured (µg/mL)	Recovery (%)
A	0.1887	0.1	0.2958	104
		0.2	0.3962	104
B	0.3448	0.1	0.4358	91.0
		0.2	0.5582	107

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Conclusion

In this application note, we demonstrated that the limit of MSA in the busulfan method could be successfully performed using the proposed USP Busulfan monograph conditions. Three commercial busulfan samples were tested and one sample was found to contain MSA impurity above the specified limit prescribed in the proposed USP monograph. We also demonstrated that this method could be executed with a modified method with comparable results. The modified method allows more time between sample preparation and analysis by better conserving sample integrity. The separation, linearity, reproducibility, and sensitivity were found to meet or exceed the proposed USP Busulfan monograph performance requirements.

References

1. Galton, D.A. Myleran in chronic myeloid leukaemia; results of treatment. *Lancet.* **1953,** *264,* 208–13.
2. Haut, A.; Altman, S.J.; Cartwright, G.E.; Wimitrobe, M.M. The use of Myleran in the treatment of chronic myelocytic leukemia. *Arch. Int. Med.* **1955,** *96,* 451.
3. Hassan, M.; Ehrsson, H. Degradation of busulfan in aqueous solution. *J. Pharm. Biomed. Anal.* **1986,** *4,* 95–101.
4. Busulfan, The United States Pharmacopeia 42nd ed., National Formulary 37th, United States Pharmacopeial Convention, Rockville MD, 2019, 623.
5. Busulfan, The United States Pharmacopeia, Pharmacopeial Forum (PF) 45(5) In-Process Revision, United States Pharmacopeial Convention, Rockville MD.
6. Thermo Scientific Dionex IonPac AS11-HC Columns Product Manual P/N 031333-09, February 2012. [online: https://assets.thermofisher.com/TFS-Assets/CMD/manuals/man-031333-ionpac-as11-hc-columns-man031333-en.pdf] (Accessed on May 2020)
7. Physical Tests, <621> Chromatography. U.S. Pharmacopeia/National Formulary (USP): Rockville, MD, USP42-NF37; p 6781.

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