(RESEARCH ARTICLE)

HPLC method for the development and validation of busulfan in pharmaceutical formulation

K Swamy Sekhar [1], Ch Venkata kishore[1], V Tejeswara Rao [2] and K. Raghu Babu [1, *]

[1] Department of Chemistry, AUEC (A), Visakhapatnam, India.
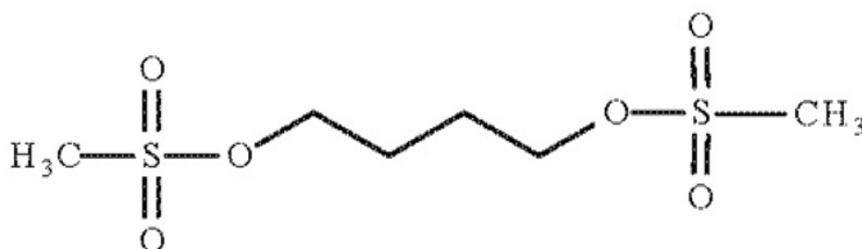[2] Department of Chemistry, MVR College, Visakhapatnam, Andhra Pradesh 530045, India.

Abstract

A validated HPLC method was developed for the determination of Busulfan (BUS) in pharmaceutical formulation.It is a new simple, accurate, precise and reproducible HPLC method has been developed for the estimation of Busulfan (1,4-butanediol dimethanesulfonate) in its inject able dosage.The method developed in High Performance Liquid Chromatographic method using suitable column (YMC Pack ODS-A (150 x 4.6) mm, 3µm). All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.The method was validated as per the ICH guidelines. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of formulations. The method developed is simple and is better than the methods reported in the literature and the method is capable to give a good detector response, the recovery calculated was within the range of 98% to 102% of the specification limits.

Keywords: Busulfan (BUS); HPLC, ICH Q2 (R1); Recovery

1 Introduction

Busulfan, a therapeutic alkane ester, is a bifunctional alkylating agent belonging to the antineoplastic therapeutic group of alkane sulfonic acid esters (1,4-butanediol dimethanesulphonate). Two labile sulphonate methane groups are bound to the opposite ends of a butyl chain. The N-7 guanine and Thiol groups of the SN2 form are known to occur in Busulfan [1].

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* Corresponding author: K.Raghu Babu
Department of Chemistry, AUEC (A), Visakhapatnam.

Specifications	Information
Identification	Busulfan
IUPAC name	1,4 – butanedioldimethanesulfonate
Molecular formula	C6H14O6S2
Molecular mass	246.306 g/mol
Category	Antineoplastic drug
Brand name	Myleran, Busulfex, Busilvex

In a liver reconstruction study done with platelets of serotonin, the serotonin was transported within the platelets. There are interstitial swelling in the lung tissues, hyper pigmentation, seizures, veno-occlusive disease (VOD),, emesis and wasting syndrome. Recently, intravenous formulations of Busulfan have been introduced on the market to bring a greater dose assurance to patients and even more on patients that are already in the hospital or in the ICU. However, the Busulfan molecules are very insoluble in water which implies that the parenteral administration only be applied above a rigidly critical threshold. The intravenous (IV) was first commercially available in its U.S. version, busulfan through the brand name Busulfex. There are three ingredients that make up this mixture: this is dissolved in N,N-dimethylacetamide (DMA)(33%) and polyethylene glycol (PEG)(400)(67%). The latter is used as a solvent. It is a clear, colorless concentrated of 6 milligrams per milliliter solution, contained in glass ampoules.

The drug is available as a tablet containing 2 mg of busulfan for oral administration and as the injection concentrate of 6 mg/mL (60 mg) of busulfan for parenteral administration for intravenous infusion. Busulfan, which is readily absorbed from the GI tract in humans when used orally, binds quickly to plasma proteins (e.g. albumin) and red blood cells, and rapidly (as in a matter of minutes) disappears from the blood. Alcohol is broken down by several enzymes when being metabolized and then its outcome comes along with a sulfur compound, depending on the alcohol level consumed and the rate at which it is metabolized. Busulfan, a form of nitrogen mustard, is a bifunctional drug that intercalates cytosine, develops sulfur bridges with DNA, binds to RNA and disrupts proteins. It is therefore capable of producing mono-adducts, intrastrand cross-links, and cross-links of DNA-protein[11-12] that are thought to play an important role in its toxic and carcinogenic effects[13-14].

2   Experimental

2.1   Chemicals and Reagents

Pure Busulfan standard and Busulfan injection were procured from a reputed reference material supplier in India. Tetrahydron, Sodium diethyl dithiocarbomatetrihydrate, N,N Dimethyl acetamide, Methanol, Water and Acetonitrile HPLC grade purchased from Merck chemicals. All the other chemicals used were of analytical grade.

2.2   Analytical conditions

Instrument : Shimadzu Class VP Binary pump LC-10ATvp, SIL-10ADvp Auto sampler, CTO-10Avp Column Temperature Oven, SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.
Mobile phase : Water, acetonitrile and tetrahydrofuran at 30:65:5 (V/V) ratio.
Column : YMC Pack ODS-A (150 x 4.6) mm, 3µm
Detection Wavelength : 280 nm
Flow rate : 1.5 mL / min
Injection volume: 20 µL
Run time : 15 minutes
Column temperature : 25° C
Sample cooling rack : 20° C
Diluent : Methanol
Run Time : 12.0 Minutes
Retention Time : 4.4 min (approximately)

2.3 Preparation of Solutions

Solution	Preparation
Mobile phase	Water, acetonitrile and tetrahydrofuran at 30:65:5 (V/V) ratio
Sodium diethyl dithiocarbomatetrihydrate stock solution (Derivatising reagent)	1000 mg of Sodium diethyl dithiocarbomatetrihydrate in to 25 mL volumetric flask, add 10ml of N, N Dimethyl acetamide sonicate to dissolve
Derivatisation Blank solution	5.0 mL of Sodium diethyl dithiocarbomatetrihydrate stock solution in to 25 mL volumetric flask, keep the solution in water bath at 60°C for 30mins and add 2 mL of diluent shake and keep solution in water bath at 60°C for 20mins
Standard stock solution	30 mg of Busulfan standard to 50 mL volumetric flask, dissolve with 25 mL of N, N Dimethyl acetamide
Derivatisation Standard solution	5.0 mL of Sodium diethyldithiocarbamatetrihydrate stock solution in to 25 mL volumetric flask, keep the solution in water bath at 60°C for 30 min and add 2 mL of standard stock solution, shake and keep solution in water bath at 60°C for 20minutes
Test sample stock solution	5.0 mL of test sample to 50 mL volumetric flask, rinse the pipette 2 times with diluent and 25ml of diluent sonicate for 10 minutes with occasional shaking
Derivatisation Test sample solution	5.0 mL of Sodium diethyldithiocarbamatetrihydrate stock solution in to 25 mL volumetric flask, keep the solution in water bath at 60°C for 30 min, add 2 mL of test sample stock solution, shake and keep solution in water bath at 60°C for 20minutes

2.4 Experimental Procedure for Method Validation

The method was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures [15-16].

2.4.1 Forced Degradation

To demonstrate stability indicating properties of the method, forced degradation was conducted by applying Thermal, UV, Acid, Alkali and Oxidation stress to the placebo and drug product.The placebo and drug product are degraded using following stress conditions.
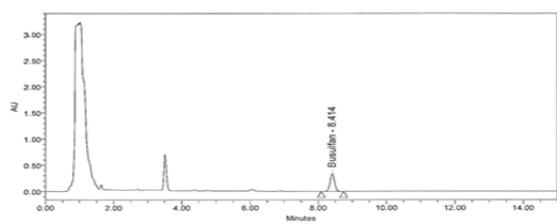
- As such (Unstressed) sample as per methodology.
- Alkali degradation: 2.0 mL of 0.2 N NaOH and keep at 30°C temperature for 60 min, and allow to cool room temperature. Neutralize with 2.0 mL of 0.2 N HCL and follow as per methodology.
- Acid degradation: 2.0 mL of 0.2 N HCl heat in water bath at 30°C for 60 min., and allow to cool at room temperature and neutralize with 2.0 mL of 0.2 N NaOH and follow as per methodology.
- Oxidation degradation: 1.0 mL of 3.0 % v/v Hydrogen Peroxide and keep it at 40°C for 30minutes, allow cooling at room temperature and following as per methodology.
- Thermal degradation: Heat at 40°C for 30minutes, allow cooling at room temperature and following as per methodology.
- UV degradation : 24 hours under UV light and follow as per methodology.
- Neutral degradation: 2.0 mL of water and keep it at 40°C for 30 minutes, allow cooling at room temperature and following as per methodology.

3 Results

The forced degradation results (peak purities of bulsulfan) are tabulated in the below table 1. Chromatograms of Samples – Unstressed, Acid, Alkali, Oxidation, Thermal, UV, Neutral conditions are exbhited from figure 1 to 7.
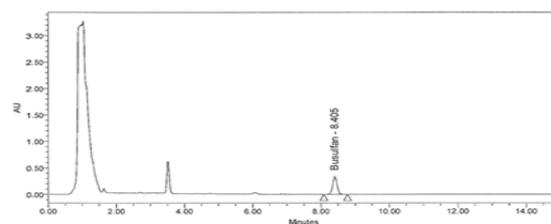
Table 1 Results of forced degradation.

Stress Condition	% Assay	% Degradation	Purity Angle	Purity Threshold
As such(Unstressed)	99.5	NA	0.681	19.281
Alkali	90.9	8.6	0.796	18.528
Acid	97.3	2.2	0.793	17.890
Oxidation	97.3	2.2	1.069	66.090
Thermal	96.9	2.6	0.678	19.149
Neutral	97.8	1.7	0.747	18.585

Figure 1 Chromatogram of Sample (Unstressed)

Figure 2 Chromatogram of Sample (Acid stressed)
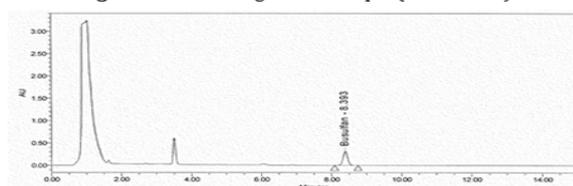
Figure 3 Chromatogram of Sample (Alkali stressed)
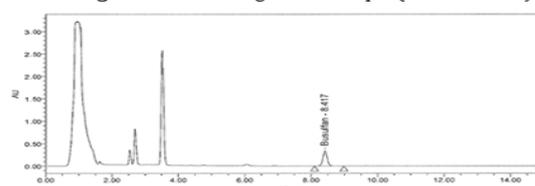
Figure 4 Chromatogram of Sample (Oxidation stressed)
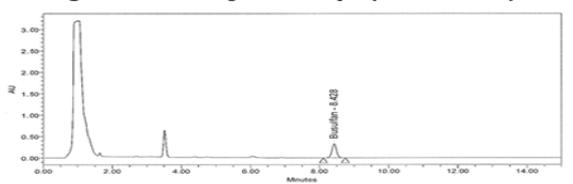
Figure 5 Chromatogram of Sample (Thermal stressed)
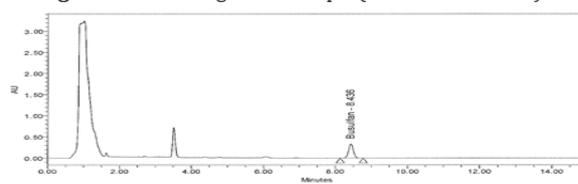
Figure 6 Chromatogram of Sample (UV stressed)
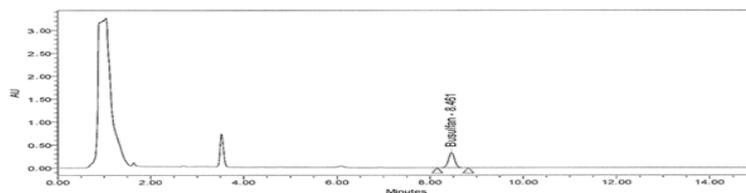
Figure 7 Chromatogram of Sample (Neutral stressed)

Chromatograms of forced degradation (Figure 1 to 7)

Stress Condition	Sample type	RT of Busulfan	Cross Reference to Chromatogram
As such (Unstressed)	Blank	N/A	1
	Sample	8.414	2
Acid	Sample	8.405	3
Alkali	Sample	8.393	4
Oxidation	Sample	8.417	5
Thermal	Sample	8.428	6
UV	Sample	8.436	7
Neutral	Sample	8.461	8

3.1   Intermediate precision

Intermediate accuracy represents differences within laboratories, such as different days, different analysts, different columns, different instruments, etc. "The definition mentioned under the terms "Intermediate Precision" as defined in USP <1225> is integrated into Ruggedness. Intermediate accuracy is developed by various analysts on different days using different columns and different equipment by performing the same exercise as system and method accuracy. Within the laboratory, the same standard Lot/Batch and sample were used. The Intermediate Precision results are tabulated in Table 2 below. The outcome of the differentiation between Process Precision and Intermediate Precision is summarized in Table 3.

Table 2 Results of Intermediate Precision.

Sample #	Retention Time (Average)	% Assay
1	8.653	101.6
2	8.692	100.6
3	8.729	101.0
4	8.733	100.3
5	8.700	99.8
6	8.670	100.2
Mean	NA	100.6
% RSD	NA	0.6

Table 3 Comparison of Method Precision and Intermediate Precision Results.

Parameter	Method Precision	Intermediate Precision
HPLC ID.	EP-QCI-013	EP-QCI-068
Column ID.	HPLCC-033	HPLCC-034
Column Sr. No.	0415219440	0415219439
Comparison of Method Precision and Intermediate precision		
Sample #	% Assay of Busulfan	
	Method Precision	Intermediate Precision
1	100.5	101.6
2	100.6	100.6

3	100.5	101.0
4	100.6	100.3
5	100.5	99.8
6	100.8	100.2
Mean	100.6	100.6
% RSD	0.1	0.6
Overall Mean	100.6	
Cumulative % RSD	0.4	

3.2   System suitability of overall validation study

The System suitability is an integral part of analytical procedure. The tests are based on the concept that the equipment, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. The system suitability results are tabulated in table 4.

Table 4 Results for System Suitability

Parameter	% RSD	Tailing Factor	Theoretical plates
System Suitability/ System Precision	0.3	1.0	16290
Specificity by diluent, placebo and known impurities	0.1	1.0	19894
Forced degradation	0.2	1.0	19380
Linearity	0.1	1.0	16047
Method Precision	0.3	1.0	16290
Intermediate Precision	0.1	1.0	16930
Accuracy (Recovery)	0.1	1.0	16047
Robustness-Flow rate: 1.3mL/minute	0.2	1.0	20283
Robustness-Flow rate: 1.7mL/minute	0.3	1.0	19156
Robustness-Column oven temperature: 23°C	0.3	1.0	20075
Robustness-Column oven temperature: 27°C	0.1	1.0	20145
Robustness-Low organic composition(637 mL)	0.1	1.0	19366
Robustness-High organic composition(663 mL)	0.1	1.0	20976
Robustness-Derivatisation temperature: 70°C	0.2	1.0	19793
Robustness-Derivatisation temperature:50°C	0.7	1.0	19952
Robustness-Derivatisation time: 10 minutes	0.4	1.0	20008
Robustness-Derivatisation time: 30 minutes	0.1	1.0	19837
Stability of Analyte in Solution (Initial)	0.3	1.0	16290
Stability of Analyte in Solution (24 Hours)	0.1	1.0	16047
Stability of Analyte in Solution (48 Hours)	0.4	1.0	16321
Filter compatibility	0.3	1.0	16290
Minimum	0.1	1.0	16047
Maximum	0.7	1.0	20976
Average	0.2	1.0	18353

4   Conclusion

A validated and appropriate analytical technique for measuring Busulfan meets the acceptance requirements for forced degradation and intermediate precision.

For determining Busulfan in pharmaceutical formulations, a simple isocratic HPLC method is developed. The findings meet and are comparable to the approval requirements and suggest that an analyst, column by column, column and equipment are accurate and rough with regard to the analyst, the instrument for its intended use. The approach can also be used in quality management routine analysis.

Compliance with ethical standards

Acknowledgments

Acknowledgments must be inserted here.

Disclosure of conflict of interest

If two or more authors have contributed in the manuscript, the conflict of interest statement must be inserted here.

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