ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Analytical Method Development and Validation for Assay Method for Quantification of Busulfan in Pharmaceutical Formulations of by HPLC Method.

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

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

ABSTRACT

A new simple, accurate, precise and reproducible HPLC method has been developed for the estimation of Busulfan (1,4-butanediol dimethanesulfonate) in its injectable dosage. A mixture water, acetonitrile and tetrahydrofuran at 30:65:5 (V/V/V) ratios were prepared and used as mobile phase. The method was validated as per the ICH guidelines. The method was validated for the determination of Assay in finished product of Busulfan Injection and the method validation parameters were evaluated for the analytical test attribute Busulfan meets the acceptance criteria. The results obtained were within the specified limits thus, this method was used for the determination of Assay in finished product of Busulfan Injection (6mg/mL).Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of formulations.

Keywords: HPLC, Busulfan, validation, mutation, anti-neoplastic.

https://doi.org/10.33887/rjpbcs/2021.12.2.5

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Busulfan was considered by previous IARC Working Groups in 1973 and 1987. Since that time, new data have become available, these have been incorporated into the Monograph, and taken into consideration in the present evaluation (1-2). Busulfan is available as a tablet containing 2 mg busulfan for oral administration, and as an injection (Busulfex) concentrate for intravenous infusion containing 6 mg/mL (60 mg) busulfan for parenteral administration.

Busulfan induced chromosomal aberrations, sister chromatid exchange, and mutations in human and rodent cells treated in vitro. It also induced sex-linked recessive lethal mutations in Drosophila, and was mutagenic to bacteria (3-4). There is sufficient evidence in humans for the carcinogenicity of busulfan. Busulfan causes acute myeloid leukaemia. There is limited evidence in experimental animals for the carcinogenicity of busulfan. Busulfan is carcinogenic to humans. Leukaemias that have developed in patients treated with busulfan (often in combination with other agents) frequently exhibit these clonal chromosomal changes (5). Busulfan-containing regimens have been widely accepted as a standard of care, and represent the most frequently used myeloablative regimens prior to HCT (6-7).

This drug used in study of platelet-transported serotonin in liver reconstruction (8). Tonicity which includes interstitial "busulfan lung", hyper pigmentation, seizures, veno-occlusive disease (9-10) (VOD), emesis, and wasting syndrome. Oral bioavailability of BUS showed very large inter-individual change (11).

ICH- international council for harmonization of technical requirements for pharmaceuticals for human use (ICH) is unique in bringing together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration.Q2 (R1) Validation of analytical procedures of methodology is document presents a discussion of the characteristics for consideration during the validation of the analytical procedures included as part of registration applications submitted within the EC, Japan and USA. This document does not necessarily seek to cover the testing that may be required for registration in, or export to, other areas of the world. Furthermore, this text presentation serves as a collection of terms, and their definitions, and is not intended to provide direction on how to accomplish validation. These terms and definitions are meant to bridge the differences that often exist between various compendia and regulators of the EC, Japan and USA. The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are Accuracy, Precision, Repeatability, Intermediate Precision, Specificity, Detection Limit, Quantization Limit, Linearity, Range (12-13).

EXPERIMENTAL

Reference Item Details

The following information was provided by the Sponsor.

Identification	Busulfan
IUPAC name	1,4 – butanedioldimethane sulfonate
Molecular formula	$C_6H_{14}O_6S_2$
Molecular mass	246.306 g/mol
Category	Antineoplastic drug
Brand name	Myleran, Busulfex, Busilvex
Introduced by	Otsuka America Pharmaceutical, Inc.
Storage conditions	Store in Cool place. Keep Container tightly closed in a dry and well-ventilated place

TEST SYSTEM

Instrument Name	:	High performance liquid chromatography
Make	:	Shimadzu
Model	:	LC-2030C
Software	:	LC Solutions

Equipment's

Following equipments were used for the study.

S. No.	Equipment	Model	Make/Supplier
1	Weighing Balance	XS205 Dual Range	Mettler Toledo
2	High Performance Liquid Chromatography	LC-2030C	Shimadzu
3	Ultrasonic cleaner	101/250	PCI Analytics
4	Micropipette	SL-1000	Rainin
5	pH Meter	PICO +	Labindia

Chemicals / Consumables

S.No.	Name	Grade	Manufacturer
1	Acetonitrile	High Performance Liquid Chromatography	Merck Limited
2	Sodium diethyl dithiocarbomatetrihydrate	High Performance Liquid Chromatography	Merck Limited
3	N,N Dimethyl acetamide	High Performance Liquid Chromatography	Merck Limited
4	Tetrahydron	Analytical Regent Grade	Merck Limited

Preparation of Solutions

All the reagents used for this proposed assay were prepared as Diluent, Standard solution (3000ppm), Sample preparation, Placebo preparation, Sample preparation (3000ppm) by using standard methods.

Injection sequence

Name of the solution	No. of Injections
Diluent	01
Derivatisation Blank	01
Standard solution	05
Test solution	02

Accuracy

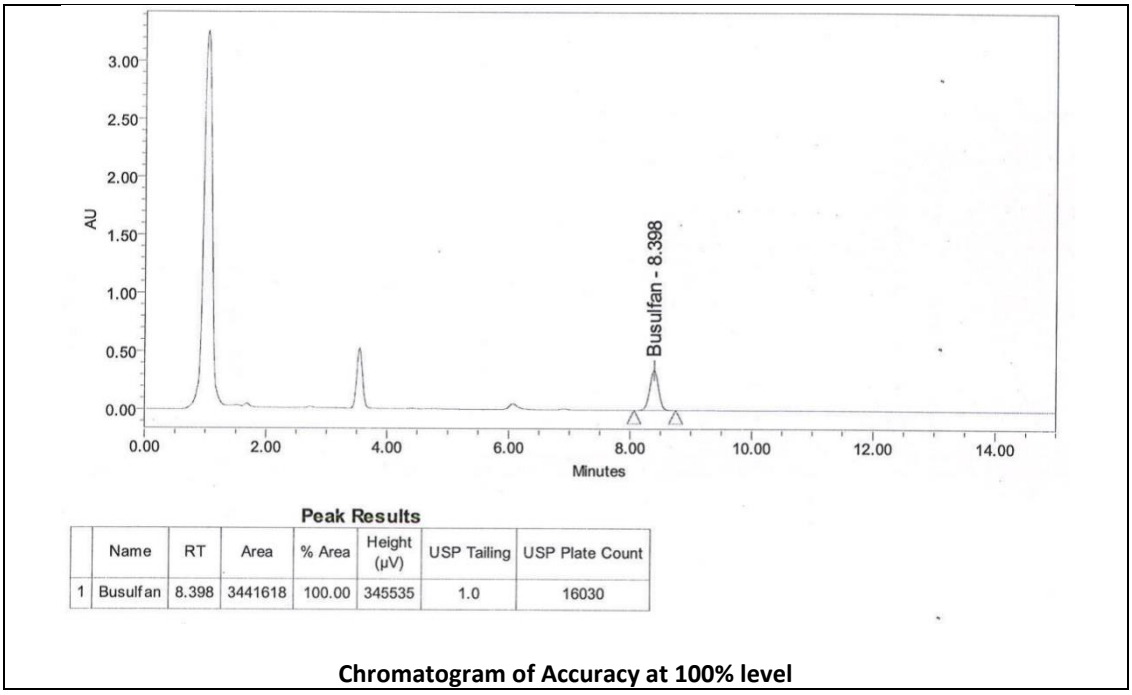
The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found. To demonstrate the accuracy of assay test method, drug substance is spiked quantitatively in to placebo from 50% to 150% of working concentration of test concentration at each level with triplicate preparation and analyzed using the test method. The accuracy results of Busulfan are tabulated in below table 8. Chromatogram of Accuracy at 100% level is exhibited below as figure.

ISSN: 0975-8585

Results of Accuracy for Busulfan

Accuracy Level	Sample #	Amount added (ppm)	Amount found (ppm)	% Recovery	Average % Recovery	% RSD
50 %	1	27.0	26.8	99.3	99.2	0.3
	2		26.7	98.9		
	3		26.9	99.6		
	4		26.8	99.3		
	5		26.8	99.3		
	6		26.7	98.9		
100 %	1	49.2	49.3	100.2	100.2	0.2
	2		49.4	100.4		
	3		49.4	100.4		
	4		49.3	100.2		
	5		49.2	100.0		
	6		49.2	100.0		
150 %	1	71.0	71.5	100.7	100.5	0.3
	2		71.5	100.7		
	3		71.5	100.7		
	4		71.1	100.1		
	5		71.5	100.7		
	6		71.1	100.1		
Overall % Recovery						100.0
Overall % RSD						0.6

Chromatogram of Accuracy



Peak Results

	Name	RT	Area	% Area	Height (µV)	USP Tailing	USP Plate Count
1	Busulfan	8.398	3441618	100.00	345535	1.0	16030

Chromatogram of Accuracy at 100% level

ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal

usage. Robustness study is performed by analyzing the standard at different conditions. The results obtained with altered conditions are compared against results obtained under normal chromatographic conditions.

Variation in Flow Rate (± 0. 2 mL/min.)

The standard was carried out by varying the flow rate of mobile phase to 1.3 mL/min. and 1.7 mL/min. in place of actual flow rate 1.5 mL/min. The results are summarized in the below. The results are tabulated in below table.

Results of robustness -Variation in flow rate for Busulfan

Injection #	Flow Rate 1.3 mL/min.		Actual Flow Rate 1.5 mL/min.		Flow Rate 1.7 mL/min.	
	RT	Area	RT	Area	RT	Area
1	9.833	3839243	8.620	3251379	7.492	2892023
2	9.829	3843384	8.619	3260248	7.493	2898533
3	9.824	3844782	8.621	3268032	7.488	2895836
4	9.822	3828026	8.624	3268763	7.485	2910064
5	9.818	3830269	8.628	3273179	7.479	2911638
Mean	NA	3837141	NA	3264320	NA	2901619
% RSD	NA	0.2	NA	0.3	NA	0.3
Tailing factor	1.0		1.0		1.0	
Theoretical plates	20283		16290		19156	

Variation in Column Oven Temperature (± 2°C)

The standard was carried out by varying the column oven temperature of 23°C and 27°C in place of actual column oven temperature 25°C. The results are tabulated in below table.

Results of robustness -Variation in Column Oven Temperature

Injection #	Column Oven Temperature 23°C		Actual Column Oven Temperature 25°C		Column Oven Temperature 27°C	
	RT	Area	RT	Area	RT	Area
1	8.769	3319801	8.620	3251379	8.654	3357455
2	8.768	3326001	8.619	3260248	8.655	3361908
3	8.767	3321444	8.621	3268032	8.653	3357755
4	8.767	3332994	8.624	3268763	8.653	3364245
5	8.767	3342848	8.628	3273179	8.654	3366094
Mean	NA	3328618	NA	3264320	NA	3361492
% RSD	NA	0.3	NA	0.3	NA	0.1
Tailing factor	1.0		1.0		1.0	
Theoretical plates	20075		16290		20145	

Variation in Organic composition (Acetonitrile content ± 2% - 637mL & 663mL)

The standard was carried out by varying the Organic composition (Acetonitrile) 637 mL and 663mL in place of actual the 650mL. The results are tabulated in below table.

ISSN: 0975-8585

Results of robustness - Variation in Organic composition

Injection #	Low Organic composition 637mL		Actual Organic composition 650mL		High Organic composition 663 mL	
	RT	Area	RT	Area	RT	Area
1	7.760	3312036	8.620	3251379	11.428	3279878
2	7.769	3314489	8.619	3260248	11.410	3284373
3	7.780	3313038	8.621	3268032	11.408	3283458
4	7.789	3315536	8.624	3268763	11.402	3284785
5	7.794	3321370	8.628	3273179	11.409	3286809
Mean	NA	3315294	NA	3264320	NA	3283861
% RSD	NA	0.1	NA	0.3	NA	0.1
Tailing factor	1.0		1.0		1.0	
Theoretical plates	19366		16290		20976	

Variation in Derivatisation Temperature (± 10°C)

The standard was carried out by varying the derivatisation temperature of 50°C and 70°C in place of actual derivatisation temperature 60°C. The results are tabulated in below table.

Results of robustness - Variation in derivatisation Temperature

Injection #	Derivatisation Temperature at 70°C		Derivatisation Temperature at 60°C		Derivatisation Temperature at 50°C	
	RT	Area	RT	Area	RT	Area
1	8.978	3601264	8.620	3251379	9.030	3743140
2	8.985	3588015	8.619	3260248	9.035	3698237
3	8.995	3585756	8.621	3268032	9.033	3672184
4	9.000	3580742	8.624	3268763	9.019	3703094
5	8.989	3579934	8.628	3273179	9.001	3687782
Mean	NA	3587142	NA	3264320	NA	3700887
% RSD	NA	0.2	NA	0.3	NA	0.7
Tailing factor	1.0		1.0		1.0	
Theoretical plates	19793		16290		19952	

Variation in Derivatisation Time (± 10 min)

The standard was carried out by varying the derivatisation Time of 10 min and 30 min in place of actual derivatisation Time 20 min. The results are tabulated in below table.

Results of robustness - Variation in derivatisation Time

Injection #	Derivatisation Time-10 min		Derivatisation Time-20 min		Derivatisation Time-30 min	
	RT	Area	RT	Area	RT	Area
1	8.911	3632833	8.620	3251379	8.861	3611110
2	8.904	3601197	8.619	3260248	8.856	3615253
3	8.899	3599984	8.621	3268032	8.853	3622831
4	8.896	3595055	8.624	3268763	8.851	3612096
5	8.893	3608676	8.628	3273179	8.850	3618418
Mean	NA	3607549	NA	3264320	NA	3615941
% RSD	NA	0.4	NA	0.3	NA	0.1
Tailing factor	1.0		1.0		1.0	
Theoretical plates	20008		16290		19837	

ISSN: 0975-8585

STABILITY OF ANALYTE IN SOLUTION

Stability of analyte in solution is evaluated for the standard and sample solutions. The standard and sample solutions are prepared and analyzed as per the analytical procedure. A portion of these solutions were preserved at room temperature and 2-8°C and analyzed at different time intervals from the time of preparations. The results are calculated from initial versus over a period of time. The results are tabulated in below tables.

Stability of Standard Solution

Time Interval	%Assay of Busulfan		% Difference	
	Room Temperature	2-8°C	Room Temperature	2-8°C
Initial	100		NA	
24 hours	100.3	100.3	-0.30	-0.30
48 hours	101.3	101.3	-1.30	-1.30

Stability of Sample Solution

Time Interval	%Assay of Busulfan		% Difference	
	Room Temperature	2-8°C	Room Temperature	2-8°C
Initial	100.5		NA	
24 hours	99.7	101.3	0.80	-0.80
48 hours	101.0	102.5	-0.50	-2.0

RESULTS

<table>
<tr><th>Validation Parameters</th><th>Acceptance Criteria</th><th colspan="5">Results</th></tr>
<tr><td rowspan="5">Accuracy</td><td rowspan="5">➢ Recovery at each level and overall average recovery of assay results should be between 98.0% and 102.0%
➢ The RSD at each level and overall RSD of % recovery should not be more than 5.0%</td><td colspan="2">Accuracy Level</td><td colspan="2">Average % Recovery</td><td>%RSD</td></tr>
<tr><td colspan="2">50 %</td><td colspan="2">99.2</td><td>0.3</td></tr>
<tr><td colspan="2">100 %</td><td colspan="2">100.2</td><td>0.2</td></tr>
<tr><td colspan="2">150 %</td><td colspan="2">100.5</td><td>0.3</td></tr>
<tr><td colspan="2">Overall % Recovery</td><td colspan="3">100.0 %</td></tr>
<tr><td>Robustness</td><td>System suitability criteria defined in test procedure should meet in each condition.
➢ The Tailing factor for Busulfan should be NMT 2.0.
➢ The relative standard deviation for Busulfan peak from five replicate injections of standard solution should be NMT 2.0 %. The theoretical plates for Busulfan peak in</td><td colspan="2">Overall % RSD</td><td colspan="3">0.6 %</td></tr>
<tr><td rowspan="4"></td><td rowspan="4"></td><td rowspan="2">Condition</td><td colspan="3">Busulfan</td></tr>
<tr><td>% RSD</td><td>Tailing factor</td><td>Theoretical plates</td></tr>
<tr><td>As such (For Flow, Temperature, Organic composition,Derivatisation temperature,Derivatisation Time)</td><td>0.3</td><td>1.0</td><td>16290</td></tr>
<tr><td>Flow rate:1.3 mL/min</td><td>0.2</td><td>1.0</td><td>20283</td></tr>
<tr><td>Flow rate:1.7 mL/min</td><td>0.3</td><td>1.0</td><td>19156</td></tr>
<tr><td>Column oven</td><td>0.3</td><td>1.0</td><td>20075</td></tr>
</table>

	standard solution should be not less than 2000.	temperature: 23°C			
		Column oven temperature: 27°C	0.1	1.0	20145
		Low organic composition(637 mL)	0.1	1.0	19366
		High organic composition(663 mL)	0.1	1.0	20976
		Derivatisation temperature: 50° C	0.7	1.0	19952
		Derivatisation temperature: 70° C	0.2	1.0	19793
		Derivatisation time: 10 min	0.4	1.0	20008
		Derivatisation time: 30 min	0.1	1.0	19837

CONCLUSION

Accuracy

The analytical test procedure is accurate for its intended use.

Robustness

The test method is robust enough as demonstrated by altering the Flow rate (± 0.2mL/min), Column oven temperature (± 2°C), Derivatisation time ((± 10min), Derivatisation temperature ((± 10°C) and Organic composition (Acetonitrile content by about ± 2%).

Stability of analyte in solution

The Standard solution is stable up to 48 hours and sample solution is stable up to 48 hours at both room temperatures as well as at 2-8°C.The data for each validation characteristic described in this report meets the acceptance criteria with respect to Specificity, Forced degradation, Stability of analyte in solution, Linearity, Method Precision, Intermediate Precision, Accuracy and Robustness.

The validation results reveal that the analytical procedure is suitable for determination of Assay of Busulfan in BusulfanInjection 60mg/10mL (6mg/mL). The method is stability indicating for determination of Assay of Busulfan in BusulfanInjection 60mg/10mL (6mg/mL).

REFERENCES

[1] IARC. Some Aromatic Amines, Hydrazine and Related Substances, N-nitroso Compounds and Miscellaneous Alkylating Agents. IARC Monogr Eval Carcinog Risk Chem Hum. 1974;4:247–252.
[2] IARC. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl. 1987;7:137–139. a.
[3] Bishop JB, Wassom JS. Toxicological review of busulfan (Myleran). Mutat Res. 1986;168:15–45.
[4] IARC. Genetic and related effects: An updating of selected IARC monographs from Volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl. 1987;6:1–729. b.
[5] Mauritzson N, Albin M, Rylander L, et al. Pooled analysis of clinical and cytogenetic features in treatment-related and de novo adult acute myeloid leukemia and myelodysplastic syndromes based on a consecutive series of 761 patients analyzed 1976–1993 and on 5098 unselected cases reported in the literature 1974–2001. Leukemia. 2002;16:2366–2378.
[6] Palmer J, McCune JS, Perales MA, Marks D, Bubalo J, et al. (2016) Personalizing busulfan-based conditioning: considerations from the American society for blood and marrow transplantation practice guidelines committee. Biology of Blood and Marrow Transplantation 22: 1915-1925.
[7] Pasquini MC, Le-Rademacher J, Zhu X, Artz A, DiPersio J, et al. (2016) Intravenous busulfan-based myeloablative conditioning regimens prior to hematopoietic cell transplantation for hematologic malignancies. Biology of Blood and Marrow Transplantation 22: 1424-1430.

[8] Lesurtel M, Graf R, Aleil B, Walther D, Tian Y, Jochum W, Gachet C, Bader M, Clavien P (2006). "Platelet-derived serotonin mediates liver regeneration". Science. 312 (5770):104–7. doi:10.1126/science.1123842. PMID 16601191.

[9] Grigg A, Gibson R, Bardy P, Szer J (1996). "Acute portal vein thrombosis after autologous stem cell transplantation". Bone Marrow Transplant. 18 (5): 949–53. PMID 8932850.

[10] Brisse H, Orbach D, Lassau N, Servois V, Doz F, Debray D, Helfre S, Hartmann O, Neuenschwander S (2004). "Portal vein thrombosis during antineoplastic chemotherapy in children: report of five cases and review of the literature". Eur. J. Cancer. 40 (18): 2659–66. doi:10.1016/j.ejca.2004.06.013. PMID 15571949.

[11] Hassan M, Ljungman P, Bolme P, Ringdén O, Syrůcková Z, Békàssy A, Starý J, Wallin I, Kållberg N (1994). "Busulfan bioavailability". Blood. 84 (7): 2144–50. PMID 7919328. Retrieved 2018-02-04.

[12] Andrew Teasdale, David Elder, Raymond W Nims, ICH Quality Guidelines: An Implementation Guide, ISBN: 978-1-118-97111-6, 2017

[13] Khagga Bhavyasri , Kaitha Manisha Vishnumurthy, Dammu Rambabu, and Mogili Sumakanth, ICH guidelines – "Q" series (quality guidelines), GSC Biological and Pharmaceutical Sciences, 2019, 06(03), 089–106.