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A SIMPLE GAS CHROMATOGRAPHY METHOD FOR THE QUANTITATIVE DETERMINATION OF RELATED IMPURITY (1,4-BUTANEDIOL) IN BUSULFAN DRUG

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ABSTRACT

A competent and significant technique has been established for the quantification of related impurity (1,4- Butanediol) in Busulfan Drug by employing Gas Chromatography furnished through Flame Ionization Detector (FID), and auto liquid sampler. Chromatographic separation accomplished on a capillary column with specifications; DB-1 phase having 30 m length, 0.53 mm i.d. and 2.65 µm thickness of the film. The methodology was validated following relevant regulatory guidances. The technique proposed was noticed to be accurate, specific, robust, stable, linear, precise, and rugged with concentration ranging from Lowest Limit of Quantitation (LLQ) level to 200% of the specification limit for 1,4-Butanediol.

Keywords: Busulfan, 1,4-Butanediol, Related Impurity, Gas Chromatography, Capillary Column.
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INTRODUCTION

Busulfan is formed by the reaction of Butanediol and Methane sulfonyl chloride in the presence of a suitable alkalizing agent and a solvent. It is a crystalline white powder, having $CH_3SO_2O(CH_2)_4OSO_2CH_3$ as molecular formula and MW of 246. Busulfan is used for the medical treatments of chronic myeloid leukemia as well as a potent alkylating agent.[1] Presently, Busulfan drug formulations are available in the market as 'Myleran', a white film-coated oral tablet having 2mg of Busulfan, and 'Busulfex' an intravenous injectable solution having 6mg/mL of Busulfan in 10 mL single vial.[2] To meet the pharmaceutical regulatory body's guidelines, it is obligatory to monitor 1,4-Butanediol impurity in Busulfan products.[3-4] 1,4-Butanediol is a related compound of Busulfan and is considered as one of the hydrolytic degradants as per the available literature.[5] Literature survey reveals thus far, there is no specific method for the quantification of 1,4-Butanediol in Busulfan drug products and it is not available in any of the pharmacopoeia official monographs.[6-11]

Hence, the easiest and specific technique by Gas chromatography with Flame Ionization Detector to determine 1,4-Butanediol in Busulfan drug was developed and validated.

EXPERIMENTAL

Chemicals and Standards

GC grade 1,4 Butanediol, Acetonitrile and Acetone with purity of almost 99.9% were procured from Merck.

Instrumentation and Chromatographic Conditions

The analysis was executed by adopting Gas Chromatography fitted with a Flame Ionization Detector (Agilent make7890A). Introduction of Samples via Split less/Split injection port and detected by FID. For

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the separation, a capillary column having a DB-1 phase with 30 m length, 0.53 mm inner dia and 2.65µm film thickness was employed. The temperature of the column oven was set at 60°C for 2 minutes and increased to 250ºC with a rate of 20º C/min, by holding at 250ºC to 10 minutes. The overall run time was 22 minutes.

The injector, as well as detector temperatures, were reserved at 210°C and 260°C correspondingly. Nitrogen (carrier gas) with a flow rate of 3.5 mL/min. Nitrogen (flow rate of 30 mL/min.) was also utilized as a makeup gas for a detector; the flow rate of hydrogen gas, as well as zero air, was 30mL/min. and 310 mL/min. correspondingly. The split ratio was adjusted to 1:2 and the injection of sample solutions was performed with an injection volume of 1µL.

Standard Solution Preparation

The impurity standard solution of 1,4 -Butanediol was prepared by using acetonitrile as a sample solvent to get a final concentration of about 0.015mg/mL.

Preparation of Test Sample Solution

The test sample solution was prepared by using acetonitrile as a sample solvent to get a nominal concentration of about 10mg/mL.

Validation of the Method

The test process validation was carried as stated by the guidelines of ICH (Q2R1), and FDA validation guidance concerning current comprehensive regulatory constraints.[12-13] As a component of test method validation, the characteristics such as accuracy, specificity, precision, ruggedness, linearity, the lower limits of both quantification and detection, solution stability, robustness, range of the test method, and system suitability were evaluated.

RESULTS AND DISCUSSION

Specificity

The Specificity of the method was confirmed by exploring sample solvent (Acetonitrile), impurity standard solution (1,4-Butanediol), test sample solution (spiked with impurity).

The chromatograms acquired for sample solution, standard solution, and spiked sample solution (with impurity standard) illustrate no intervention with impurity of Busulfan (1, 4-Butanediol) peak and thus the method is specific (Figs.-3, 4 and 5).

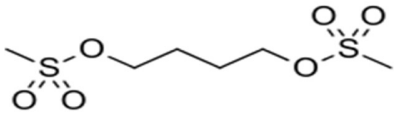

Fig.-1: Structure of Busulfan

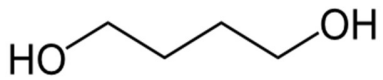

Fig.-2: Structure of 1,4-Butanediol

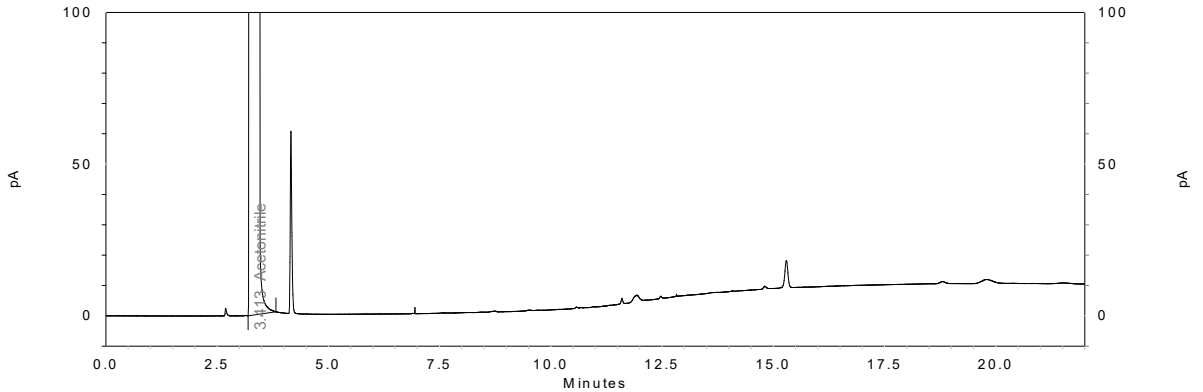

Fig.-3: Chromatogram of Sample Solvent (Blank)

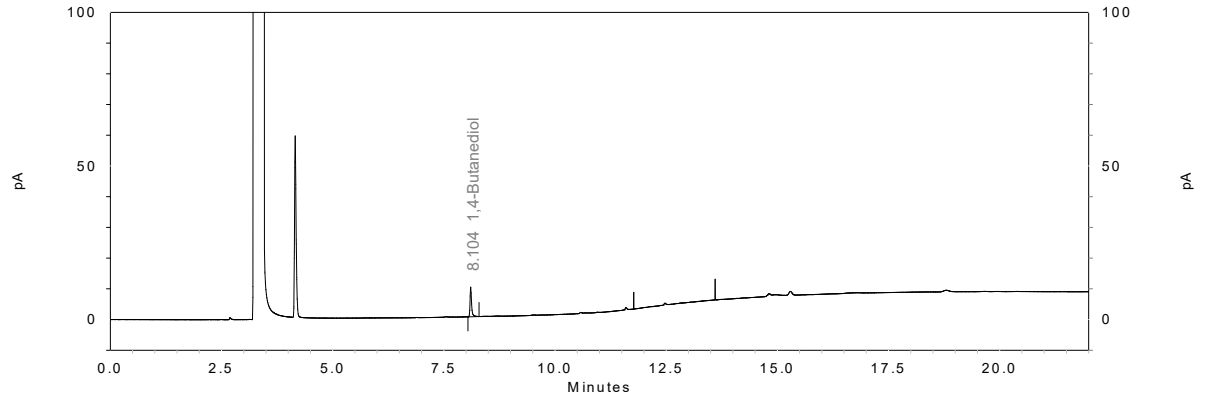

Fig.-4: Chromatogram of Impurity Standard (1,4-Butanediol)

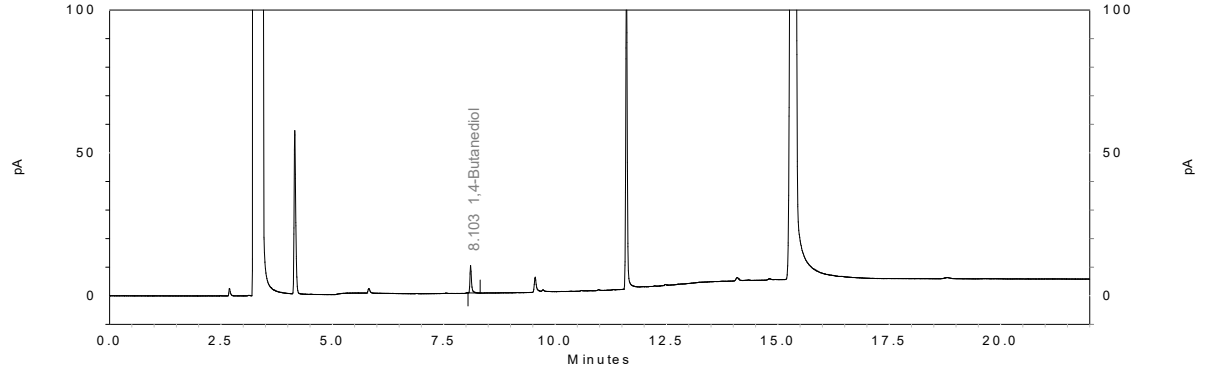

Fig.-5: Chromatogram of Spiked Test Sample

Precision

Repeatability of the system was estimated by introducing 6 replicate injections of an impurity standard solution (1, 4 -Butanediol). The RSD (%) was 0.0% and 0.5% for both the responses (i.e., retention time and peak area), which specifies the procedure's repeatability.

Reproducibility was verified from (n=6) successive results of quantification obtained from the homogeneous single lot test sample. The (%) RSD for quantifying the result of 1,4-Butanediol impurity from the samples was noticed to be 0.5%, which specifies the reproducibility of the technique.

Ruggedness

The ruggedness of the procedure was assessed from spiked sample analysis through a diverse instrument, column and analyst on different day/s. The RSD (%) for the quantified results of 1,4-Butanediol impurity acquired from 6 verifications (inter precision) was 0.6% along with the cumulative RSD (%) for twelve verifications (both intra and inter precision) establishes 1.9% and thus the technique is rugged.

Linearity

Prepared, various concentration levels of standard solutions ranging from LLQ level to about 200% level of the nominal concentration for 1,4-Butanediol. A linear correlation and regression were noticed among the concentrations and peak area responses of 1,4-Butanediol in the precise range (lower quantifiable limit to 200% of nominal concentration), tabulated in Table-1, demonstrating the linearity of the procedure (Fig.-6).

Lowest Limit of Detection and Quantification (LLD and LLQ)

LLD and LLQ were derived from residual standard deviation and linearity slope. The derived LLD and LLQ values for impurity (1,4-Butanediol) were 77 ppm (0.0077%) and 253 ppm (0.0253%) respectively and analyzed the respective concentration level solutions. The RSD (%) for peak area responses at LLQ concentration for 6 demonstrations was 1.6%, and a discrete visible peak noticed at LLD concentration, indicates the sensitivity of the methodology.

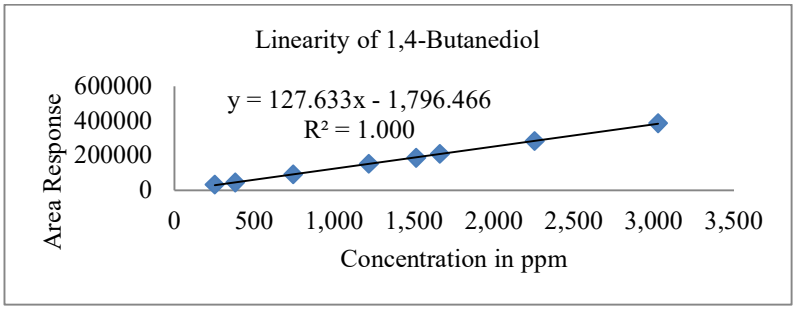

Fig.-6: Linearity Curve for 1,4-Butanediol

Table-1: Linearity of 1-4 Butanediol

% Level	Concentration(µg/mL)	Area Response
LLQ	252.1148	32506
25	379.6552	46147
50	741.5140	92375
80	1216.0830	154039
100	1512.6886	187853
120	1660.9914	211682
150	2254.2026	284057
200	3025.3771	386376
Correlation (r)		1.000
Regression (R^2)		1.000
Slope		127.633
y-intercept		-1796.466
% y-intercept		-0.6

Accuracy (Recovery) and Range

Recovery was executed by evaluating triplicate samples spiked with 1,4- Butanediol impurity at LLQ, 50%, 100%, 120%, 150%, and 200% level. The percentage of recovery was evaluated from the impurity amount added and recovered. On the whole mean % recovery was 100.2% (RSD= 1.2%). The results put in Table-2, signify the recovery effectiveness of the procedure. The range for 1,4-Butanediol recovered from the sample matrix was accurate, precise, and linear from lesser to upper levels. The data are tabulated in Table-3, indicating the range of test methods.

Table-2: Accuracy and Range

% Level	Sample	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean % Recovery	% RSD
LLQ	1	0.0254	0.0253	99.6	99.6	1.2
	2	0.0254	0.0256	100.8		
	3	0.0254	0.0250	98.4		
50%	1	0.0749	0.0738	98.5	99.5	0.8
	2	0.0749	0.0747	99.7		
	3	0.0749	0.0750	100.1		
100%	1	0.150	0.151	100.7	100.4	1.0
	2	0.150	0.149	101.3		
	3	0.150	0.152	100.4		
150%	1	0.225	0.222	98.7	100.6	1.7
	2	0.225	0.228	101.3		
	3	0.225	0.229	101.8		
200%	1	0.299	0.298	99.7	100.9	1.2
	2	0.299	0.302	101.0		
	3	0.299	0.305	102.0		
Cumulative Mean % Recovery					100.3	
Cumulative Mean % RSD					0.6	

Table-3: Range

% Level	Amount Added(µg/mL)	Amount Found(µg/mL)
LLQ	0.0254	0.0253
50	0.0749	0.0745
100	0.150	0.151
150	0.225	0.226
200	0.299	0.302
Correlation (r)		1.000
Regression (R^2)		1.000
Slope		1.011
y-intercept		-0.001
% y-intercept		-0.7

Robustness

Robustness was evaluated from the test sample spiked with impurity standard solution (1, 4-Butanediol), at 100% level using diverse experimental provisions of chromatographic frontier-like, carrier gas flow rate (3.3mL/min, 3.5mL/min, and 3.7mL/min), initial column oven temperature (45ºC/min, 50ºC/min and 55ºC/min), injector temperature (205º C, 210º C, and 215º C) and detector temperature (255º C, 260º C, and 265º C). The tabulated data (Table-4) shows the method was robust as there are no deviations in chromatographic conditions.

Table-4: Robustness

Chromatographic Condition	Symmetry Factor	%RSD (Area Response)	% Recovery (1,4-Butanediol)
Original conditions	1.4	0.5	100.8
Increase in Flow	1.2	0.7	102.1
Decrease in Flow	1.4	0.6	101.3
Increase in Column oven temperature	1.3	0.5	99.8
Column oven (Decrease) temperature	1.1	0.9	98.7
Injector temperature (Increase)	1.4	1.2	99.3
Injector temperature (Decrease)	1.3	0.9	100.8
Detector temperature (Increase)	1.3	1.1	101.2
Detector temperature (Decrease)	1.4	0.9	102.9

Stability of Analytical Solution

Solution stabilities of both standards, as well as test sample solutions, were assessed for successive time intervals by the side of ambient temperature (25ºC±2ºC). The differences (%) for peak area response among initial and respective time interval was determined. The difference in the peak area response since initial to 48 hours acquired for standard and test was -0.7% and -0.3% correspondingly, which designates that analytical solutions were more stable up to 48 hours.

System Suitability

System suitability was examined for the impurity standard (1,4-Butanediol) solution in every validation study factor. Peak symmetry factor and RSD (peak area response) were assessed for impurity standard (1,4-Butanediol) solution and found RSD (<10.0%) and symmetry factor (<2.0), confirms that the correctness of the analytical method.

Method Development

Since 1,4 Butanediol has been an alcoholic and an organic compound, the HPLC method will not be suitable for trace level quantification of impurity due to poor sensitivity. Thus, the preferred GC-FID method is more appropriate due to higher sensitivity towards organic compounds and can quantify the impurity at trace levels. The liquid sample injection method has been preferred instead of the headspace method to

avoid degradation due to sample heating. Different chromatography variables were applied to achieve proper separation and recovery of impurity from the sample matrix. The variables comprise; selection of suitable sample solvent (Acetone, N, N Dimethylformamide, Methanol, Acetonitrile), columns with the diverse stationary phase and diverse dimensions (polar column: HP-Innowax, mid polar column: AT-624 and non-polar columns: DB-5, DB-1) and Gas Chromatographic conditions (temperature: oven, Injection port, and FID; flow: carrier gas and fuel gas; and split ratio).
Eventually, based on the solubility of the sample in acetonitrile, the technique was optimized- separation and recovery of impurity from the typical sample matrix using a capillary column having a non-polar stationary phase with ideal Gas Chromatographic conditions.

CONCLUSION

A simple, highly sensitive, and suitable GC-FID technique for the quantification of related impurity (1,4-Butanediol) in Busulfan drug was developed and validated as per the requirement. The proposed methodology was found to be capable enough to detect and quantify the potential degrading impurity(1,4-Butanediol) in Busulfan drug products. The selectivity and reproducibility study indicates the consistent nature of the method throughout the stable life cycle of the product. The method was found to be sensitive at the lowest concentration. The test method precision, linearity and accuracy were established by covering a wide range from LLQ to 200% specification limit which will be helpful to apply the method even with increased specification limits for impurity based on the stability trend of the product. The procedure efficaciously was appropriate to quantify the related impurity (1,4-Butanediol) in Busulfan drug products. Henceforth this method can be implemented in a quality control environment to monitor and control the related impurity (1,4-Butanediol) in Busulfan drug products.

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