Journal of Chromatographic Science, 2016, 1–6
doi: 10.1093/chromsci/bmw117
Article

Article

Gas Chromatographic Method for the Quantitative Determination of a Hydrolytic Degradation Impurity in Busulfan Injectable Products

H. Ramakrishna Reddy[1,*], N. Chandrasekhar[2], and C. S. Karigar[3]

[1]Research and Development Centre, Bharathiar University, Coimbatore 641046, India, [2]Research and Development Centre, Department of Chemistry, Shridevi Institute of Engineering and Technology, Tumkur 572106, Karnataka, India, and [3]Department of Biochemistry, Bangalore University, Bangalore 560001, Karnataka, India

*Author to whom correspondence should be addressed. Email: rkreddy_h@yahoo.co.in

Received 17 January 2016; Revised 4 May 2016

Abstract

An efficient and stability-indicating method has been developed and validated for the quantitative determination of tetrahydrofuran (THF), a hydrolytic degradation impurity, in Busulfan injectable pharmaceutical products by using gas chromatograph equipped with a liquid autosampler and a flame ionization detector. The chromatographic separation was performed on a fused silica capillary (Stabilwax; 60 m length × 0.32 mm i.d., 0.5 µm film thickness) column. The methodology was validated in accordance with regulatory guidelines. The proposed method was found to be specific, stable, precise, linear, accurate, robust, and rugged in the concentration range from 4 to 1,080 ppm for THF. The developed method was successfully applied to determine the THF content in Busulfan injectable pharmaceutical products.

Introduction

Busulfan is a white crystalline powder with a molecular formula of $CH_3SO_2O(CH_2)_4OSO_2CH_3$ and a molecular weight of 246 (Figure 1). Busulfan is a bifunctional alkylating antineoplastic agent. It belongs to the class of alkyl sulfonates with the chemical name 1,4-butanediol dimethanesulfonate. Busulfan is considered as the mainstay of the chemotherapeutic treatment of chronic myeloid leukemia and similar disorders (1, 2).

Initially, Busulfan was introduced as an oral drug. Later an intravenous busulfan injectable formulation was developed to overcome the dosing-related issues. The injectable formulation of busulfan is currently available in the market as BUSULFEX®, as a clear, colorless, sterile solution in 10 mL single-use vials. Each vial contains 60 mg (6 mg/mL) of busulfan as an active ingredient. Busulfan is dissolved in *N*,*N*-dimethylacetamide, 33% (v/v) and polyethylene glycol 400, 67% (v/v). The solubility of busulfan in water is 0.1 g/L and the pH of BUSULFEX diluted to ~0.5 mg/mL in 0.9% sodium chloride (USP) or 5% dextrose (USP) as recommended for infusion reflects the physiological pH ranges (1, 2).

The degradation pathway of busulfan in aqueous solution has been investigated (1, 3–6). Busulfan yields 4-hydroxybutyl methanesulfonate and methanesulfonic acid as degradation products. Furthermore, the unstable intermediate 4-hydroxybutyl methanesulfonate undergoes cyclization to tetrahydrofuran (THF) and 1,4-butanediol. The level of formation of THF depends on the rate of cyclization of 4-hydroxybutyl methanesulfonate.

Among the above-mentioned degradation products depicted in Figure 2, THF is one of the toxic impurities listed under the residual solvents class II. The permitted daily exposure of THF is 7.2 mg (not more than 720 ppm) and the impurity level with respect to maximum daily dose of Busulfan (224 mg) is 3.2%.

Hence, according to International Conference on Harmonization (ICH) guidelines (7), it is obligatory to monitor the THF level in the drug product Busulfan injection (8, 9).

Literature survey reveals that thus far (10–14), Hassan and Ehrsson reported a gas chromatographic method which includes an electron capture detector and a flame ionization detector (FID) for the identification of THF in the hydrolysis degradation study for busulfan

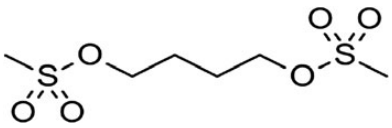

Figure 1. Structure of Busulfan.

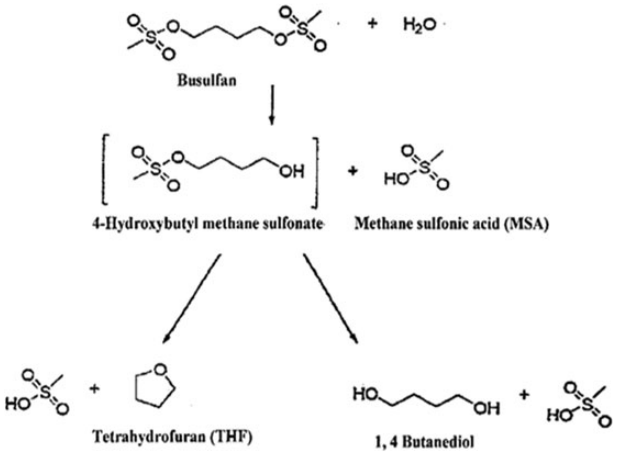

Figure 2. Hydrolytic degradation pathway of Busulfan.

drug substance. However, there is a significant need for a specific methodology for the quantitative determination of THF in busulfan injectable drug formulations.

This paper presents a simple and stability indicating GC-FID (gas chromatograph equipped with flame ionization detector, FID) method for the quantitative determination of THF in Busulfan injectable pharmaceutical products. The proposed method is sensitive at lower concentration (4 ppm) of THF. The specificity and stability of the method was proved from the spiking and degradation study. The precision, linearity and accuracy of the method was demonstrated for THF in the concentration range from the limit of quantitation (~25 ppm) level to 150% (~1080 ppm) of nominal concentration (720 ppm).

Experimental

Chemicals and standards

THF standard (GC grade standard with purity 99.98% obtained from Sigma-Aldrich), acetone (GC grade solvent with purity 99.8 obtained from Merck), water (MilliQ), dimethylacetamide (GC grade solvent with purity 99.5% obtained from Merck), 1,4-butanediol (GC grade solvent with purity 99.8% obtained from Sigma-Aldrich) and polyethylene glycol (PEG-400) (GC grade solvent obtained from Merck) were used in the experiment.

Instrumentation and chromatographic conditions

A gas chromatograph equipped with a liquid auto sampler and an FID (7890A, Agilent Technologies with EZ-Chrome software) was used to perform the analysis. Samples were introduced in a split/splitless injection port and the detection was performed by means of FID. A capillary (Stabilwax; 60 m length, 0.32 mm inner diameter and 0.5 µm film thickness) column (Restek) was used for separation. The column oven temperature was programmed with an initial temperature of 50°C for 15 min and then increased to 230°C at the rate of 20°C/min, held at 230°C for 19 min and then increased to 240°C at the rate of 20°C/min, held at 240°C for 26.5 min. The total run time was 70 min. The injector and detector temperatures were kept at 270 and 275°C, respectively. Nitrogen was used as a carrier gas. The gradient carrier gas flow rate was programmed with an initial flow rate of 1 mL/min, up to 20 min, and increased to 2 mL/min, up to 70 min. Nitrogen gas was used as a makeup gas for FID and the flow rate was 20 mL/min, whereas for hydrogen gas and zero air flow rate was 30 and 300 mL/min, respectively. The split ratio was set at 50:1 and the purge flow rate was 15 mL/min. The sample solutions were injected by a liquid autosampler of GC with an injection volume of 1 µL.

Standard and test sample solution preparation

The standard solution of THF was prepared in acetone to obtain a final concentration of ~0.144 mg/mL, and the test sample solution was prepared in acetone to obtain a final concentration of 200 mg/mL.

Validation of the method

Validation of the method was performed as per the ICH (Q2R1), USP<1225> validation guidelines and also other guidelines with respect to global regulatory requirement (7–9, 15–20). As part of method validation, parameters such as specificity, forced degradation, precision, ruggedness, linearity, accuracy, lowest detection limit (LDL) and lowest quantitation limit (LQL), robustness, solution stability, system suitability, and range of the test method were evaluated.

Specificity and forced degradation

Specificity of the method was evaluated by analyzing blank (acetone), standard solution (THF), test sample solution, placebo solution (sample without drug), and test sample solution spiked with standard.

Forced degradation was performed by subjecting the sample to hydrolytic (the sample was treated with water at 50°C for 1 h), thermal (the sample was kept in hot air oven at 80°C for 4 h) and photolytic (the sample was exposed to 1.2 million Lux hours of cool fluorescent light and an integrated near-UV energy exposure of 200 W h/m^2 simultaneously in a photostability chamber maintained at 25°C) degradation under stressed conditions.

Precision (repeatability and reproducibility)

System precision (repeatability) was evaluated by injecting six replicate injections of THF standard solution. For method precision (reproducibility), six sample solutions ($n = 6$) were prepared and analyzed. The relative standard deviation (RSD) for area response and retention time of standard in system precision, and concentration of THF in sample were calculated.

Ruggedness

Ruggedness of the method was evaluated by performing the sample analysis using different instrument, column, by different analyst on different day. The cumulative RSD was calculated for 12 sample determinations ($n = 12$) obtained from intra- and inter-precision.

Linearity

From a stock standard solution of THF, different concentrations of standard solution ranging from the lowest level (3.999 ppm) to ~150% level (1,079.784 ppm) of the nominal standard concentration (720 ppm) were prepared. The peak area responses were plotted against the respective concentrations and the obtained data were subjected to linear regression analysis.

Lowest detection limit and lowest quantitation limit

The lowest detection limit and the lowest quantitation limit were derived from the linearity slope and residual standard deviation. The derived LDL and LQL for THF were 8 and 25 ppm, respectively. The solutions of appropriate concentration were prepared and injected.

Accuracy (recovery) and range

Accuracy test was performed by analyzing the samples spiked with a known amount of THF standard at LQL, 50, 100, 120, and 150% levels. At LOQ and 150% level, six samples were prepared and triplicate samples were prepared for other levels. The % recovery was calculated from the amount of standard added and the amount of standard recovered.

The range of the test method was derived based on the precision, linearity and recovery obtained from the accuracy study in the presence of test sample matrix ranging from lower to upper limits.

The linearity of the test method across the range was drawn from the mean values of "amount added" and "amount recovered" at each level.

Robustness

For evaluation of robustness, standard solution of THF, sample spiked with THF standard at 100% level, was prepared and analyzed under different experimental conditions by varying different chromatographic parameters; initial carrier gas flow rate (0.8, 1 and 1.2 mL/min), initial column oven temperature (45, 50 and 55°C/min), injector temperature (265, 270 and 275°C) and detector temperature (270, 275 and 280°C). The RSD for area response, symmetry factor and recovery were evaluated under each varied method condition.

Stability of analytical solutions

For evaluation of analytical solution stability, THF standard solution and test sample solution were prepared and analyzed for different time intervals at ambient temperature (25 ± 2°C). The % difference for peak area responses between initial and each time interval was evaluated.

System suitability

The system suitability was evaluated for THF standard solution in all of the parameters of the validation study. RSD for peak area responses and USP symmetry factor were calculated.

Results

Method validation

Specificity and forced degradation

The obtained chromatograms for standard solution, sample solution, sample solution spiked with standard and degraded samples showed no interference with THF peak, proving that the method is specific (Figures 3 and 4).

The sample found degraded under thermal-stressed (~30% degradation; THF % level observed is 0.74%) and Neutral-stressed (~27% degradation; THF% level observed is 0.67%) conditions. However, no unknown impurities were found that could have interfered with the THF peak, proving that the method is stability indicating (Figures 5–7).

Precision (repeatability and reproducibility)

System precision (repeatability) was determined from the area response and retention time of the THF peak obtained from six repeatable standard determinations. The %RSD was 0.5 and 1.8 for the retention time and peak area response, respectively, which indicates the repeatability of the method.

Method precision (reproducibility) was confirmed from the six reproducible results of quantification acquired from the single lot of homogeneous test sample. The % RSD for the quantified results of THF from samples found 1.1%, which indicates the reproducibility of method.

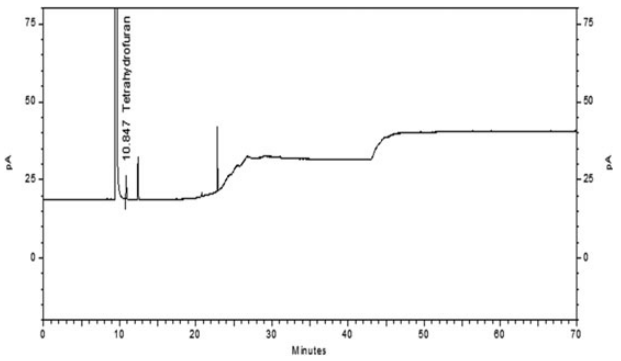

Figure 3. Chromatogram of THF standard solution.

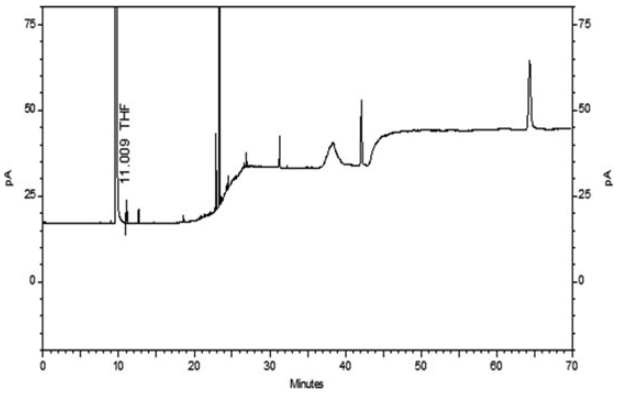

Figure 4. Chromatogram of sample spiked with THF standard.

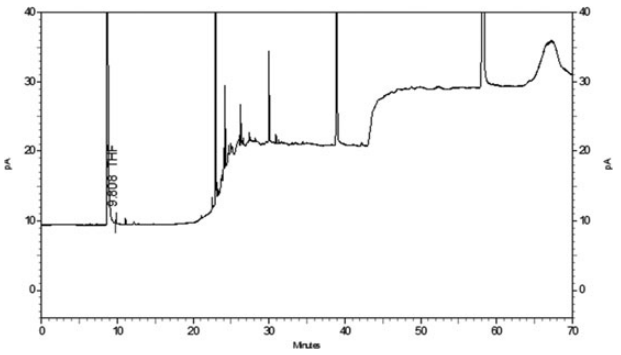

Figure 5. Chromatogram of neutral stressed sample.

Ruggedness

The %RSD for the quantified results of THF obtained from 6 determinations (inter-precision) was 1.5 and the cumulative %RSD for 12 determinations (obtained from intra- and inter-precision) was found 1.2, indicating that the method is rugged.

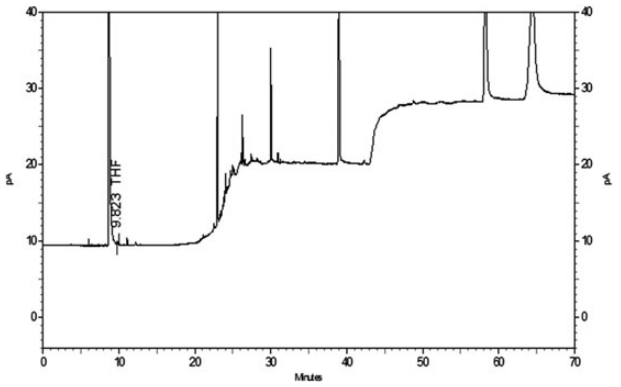

Figure 6. Chromatogram of thermal stressed sample.

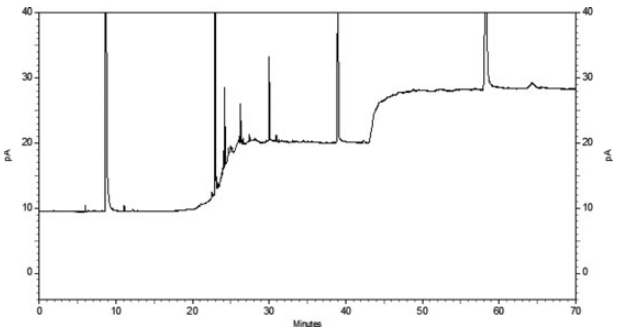

Figure 7. Chromatogram of photolytic stressed sample.

Linearity

A linear correlation and regression was found between the peak area responses and concentrations of THF in the specified range (lowest quantitation limit to 150% of nominal concentration). The data presented in Table I indicate the linearity of method (Figure 8).

Lowest detection limit and lowest quantitation limit

The LDL and the LQL were evaluated for the derived LDL and LQL for THF (8 and 25 ppm). The % RSD for the THF peak area response at LQL concentration obtained for six determinations was 2.0%, and a distinct visible peak was observed at LDL concentration, proving that the method is sensitive (Figure 9).

Accuracy (recovery) and range

Accuracy test was performed by analyzing the spiked samples at LQL, 50, 100, 120 and 150% level. The overall mean % recovery was 98.7% (RSD = 0.9%). The results tabulated in Table II indicate the recovery efficiency of the method.

The range of THF concentration recovery from sample matrix was found linear, accurate and precise at lower and higher levels. A linear graph was drawn between the mean values of "amount added" and "amount recovered" from the LQL to 150% level (Figure 10).

Robustness

The robustness of the test method was evaluated for minor but deliberate variations in the method conditions. The variation factors such as initial carrier gas flow rate (0.8, 1 and 1.2 mL/min), initial column oven temperature (45, 50 and 55°C/min), injector temperature (265, 270 and 275°C) and detector temperature (270, 275 and 280°C) were considered. The effect of variations in method conditions was evaluated for area response, symmetry factor and recovery. The data represented in Table III indicates that the methodology was not affected from the deviations in chromatographic conditions and thus the reliability of the method.

Table I. Linearity Curve for THF

% Level	Concentration (ppm)	Peak area response
0.5	3.9992	958
1.25	8.9982	2,863
2.5	17.9964	5,032
5	35.9928	11,342
10	71.9856	22,467
25	179.964	52,621
50	359.928	109,853
80	575.8848	174,628
100	719.856	216,952
120	863.8272	250,439
150	1,079.784	303,517
Correlation coefficient (r)		0.999
Regression coefficient (R^2)		0.998
Slope		287.98439
y-intercept		2,026.08214
% y-intercept		0.93

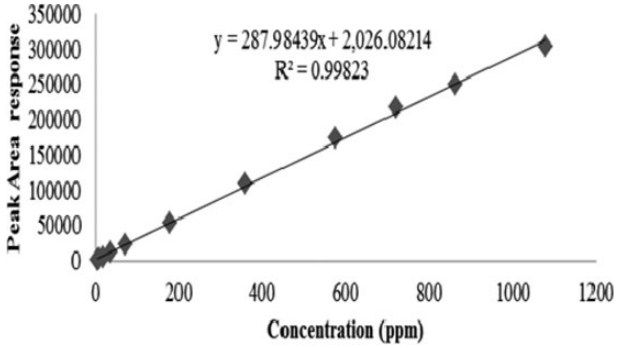

Figure 8. Linearity curve for THF peak area response.

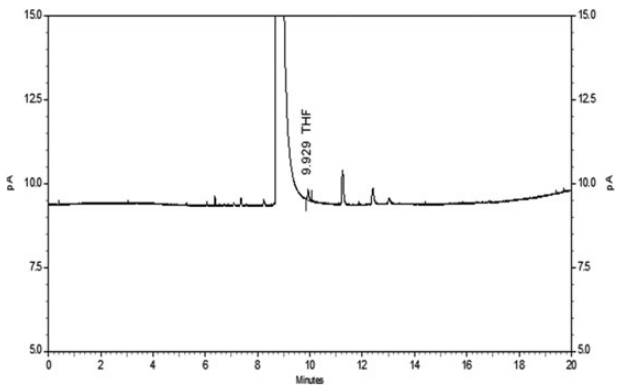

Figure 9. Chromatogram of limit of quantitation.

Stability of standard and test sample solutions

The standard and test sample solution stability was evaluated for different time intervals at ambient temperature (25 ± 2°C). The %

Table II. Accuracy and Range

Accuracy level	Sample	Amount added (ppm)	Amount recovered (ppm)	% Recovery	Mean % recovery	% RSD
LOQ	1	24.641	24.189	98.2	98.0	1.1
	2	24.641	23.739	96.3		
	3	24.641	24.488	99.4		
	4	24.641	24.009	97.4		
	5	24.641	24.395	99.0		
	6	24.641	24.015	97.5		
50%	1	362.365	359.029	99.1	98.9	0.6
	2	362.365	355.997	98.2		
	3	362.365	359.956	99.3		
100%	1	724.73	717.746	99.0	99.3	0.2
	2	724.73	721.038	99.5		
	3	724.73	719.398	99.3		
120%	1	869.676	854.556	98.3	99.4	1.1
	2	869.676	872.880	100.4		
	3	869.676	865.866	99.6		
150%	1	1,087.095	1,065.026	98.0	98.6	0.7
	2	1,087.095	1,062.713	97.8		
	3	1,087.095	1,079.688	99.3		
	4	1,087.095	1,074.613	98.9		
	5	1,087.095	1,071.520	98.6		
	6	1,087.095	1,080.357	99.4		
		Overall mean % recovery			98.7	
		Overall mean % RSD			0.9	

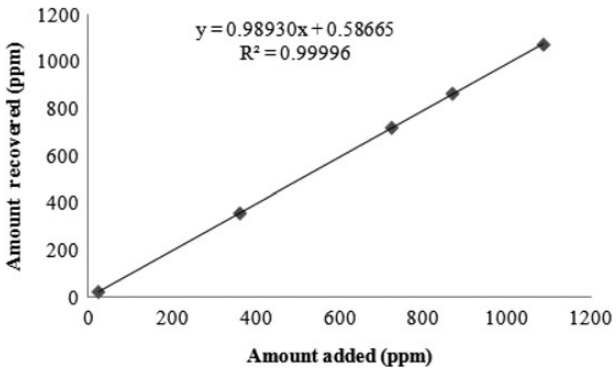

Figure 10. Linearity curve for THF recovery.

Table III. Robustness

Chromatographic condition	USP symmetry factor	%RSD for THF peak area response	% Recovery of THF from spiked sample
Original conditions	1.1	1.8	99.3
Increase in flow	1.0	1.4	99.6
Decrease in flow	1.1	1.1	99.4
Increase in column oven temperature	1.6	1.2	99.5
Decrease in column oven temperature	1.2	1.1	99.3
Increase in injector temperature	1.4	1.1	99.1
Decrease in injector temperature	1.3	1.2	99.3
Increase in detector temperature	1.4	1.1	99.4
Decrease in detector temperature	1.2	1.8	99.2

difference for peak area responses between initial and each time interval was calculated.

The % difference in the peak area responses from initial to 48 h obtained for standard and test solution was −0.8 and 1.2%, respectively, which indicates that the analytical solutions were stable at least for 48 h.

System suitability

For all parameters of validation, the specified system suitability parameters: RSD for peak area response and USP symmetry factor were evaluated for THF standard solution and found % RSD <10.0% and USP symmetry factor <2.0, proving that the suitability of analytical system.

Discussion

Method development

Tetrahydrofuran impurity by nature is a solvent and estimation by HPLC will be incompatible and inaccurate. Thus, the GC-FID methodology is an appropriate technique. As the busulfan injectable product formulation contains 67% of PEG-400, the separation of analyte peak from the sample matrix is a typical task. The head space GC method was tried to avoid the sample matrix. However, the headspace method was found to be inappropriate due to the degradation of sample during heating in the head space oven. Hence, the GC method with liquid injection sampler has been preferred.

To develop a stable GC-FID method for the estimation of THF, different chromatographic factors were evaluated. These factors include the selection of a suitable organic solvent for standard and sample preparation (dimethylsulfoxide, dimethylformamide, dimethylacetamide, methanol, ethanol, acetone), columns with different stationary phase and different dimensions (nonpolar columns like DB-5, HP-1; mid-polar columns like DB-624 and polar columns like HP-WAX, DB-FFAP, Stabilwax) and GC chromatographic (injector, detector and oven temperature program) conditions.

The final methodology was optimized based on the solubility of standard and sample in acetone, separation of analyte from the sample matrix and good recovery using the Stabilwax column under ideal GC chromatographic conditions.

Conclusion

A gas chromatography with FID (GC-FID) method for the determination of THF, a hydrolytic degradation impurity in busulfan injectable pharmaceutical product, was developed and validated as per analytical quality-by-design approach. The method was found to be specific, sensitive, linear, accurate and robust. The developed methodology was successfully applied to determine the degradation impurity, THF, in test samples of busulfan injectable product. Hence, this methodology can be adopted by quality control laboratories in the regular and stability analysis for the determination and control of THF impurity in busulfan injectable formulation products.

Acknowledgments

The authors are thankful to resource providers and anonymous reviewers for their contributions which helped to carry out this work.

References

1. Karstens, A., Krämer, I.; Stability of busulfan injection solution (Busilvex, Busulfex) in B/Braun Injekt syringes; *Pharmazie*, (2006); 61: 845–850 (article in German).
2. *Busilvex: summary of product characteristics*. European Medicines Agency, London, (2014). http://www.medicines.org.uk/emc/medicine/12967/SPC/ (accessed February 5, 2016).
3. Hassan, M., Ehrsson, H.; Degradation of busulfan in aqueous solution; *Journal of Pharmaceutical and Biomedical Analysis*, (1986); 4: 95–101.
4. Feit, P.W., Rastrup-Andersen, N.; 4-Methanesulfonyloxybutanol: hydrolysis of busulfan; *Journal of Pharmaceutical Sciences*, (1973); 62: 1007–1008.
5. Houot, M., Poinsignon, V., Mercier, L., Valade, C., Desmaris, R., Lemare, F., *et al*.; Physico-chemical stability of busulfan in injectable solutions in various administration packages; *Drugs R D*, (2013); 13: 87–94.
6. Karstens, A., Krämer, I.; Chemical and physical stability of diluted busulfan infusion solutions; *European Journal of Hospital Pharmacy Science*, (2007); 13: 40–47.
7. International Conference on Harmonization (ICH); *Q2 (R1) Validation of Analytical Procedures: Text and Methodology* (2005). http://www.ich.org/products/guidelines/quality/article/quality-guidelines (accessed February 5, 2016).
8. International Conference on Harmonization (ICH); *Q3B (R2), Impurities in New Drug Products* (2006). http://www.ich.org/products/guidelines/quality/article/quality-guidelines (accessed February 5, 2016).
9. International Conference on Harmonization (ICH); *Q3C (R5), Guideline for Residual solvents* (2011). http://www.ich.org/products/guidelines/quality/article/quality-guidelines (accessed February 5, 2016).
10. Andersson, B.S., Kashyap, A., Gian, V., *et al*.; Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: a phase II study; *Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation*, (2002); 8: 145–154.
11. Galton, D.A.; Myleran in chronic myeloid leukaemia; results of treatment; *Lancet*, (1953); 264: 208–213.
12. Santos, G.W.; The development of busulfan/cyclophosphamide preparative regimens; *Seminars in Oncology*, (1993); 20: 12–16.
13. Hassan, M., Ehrsson, H.; Gas chromatographic determination of busulfan in plasma with electron-capture detection; *Journal of Chromatography*, (1983); 277: 374–380.
14. Vassal, G., Re, M., Gouyette, A.; Gas chromatographic-mass spectrometric assay for busulfan in biological fluids using a deuterated internal standard; *Journal of Chromatography*, (1988); 428: 357–361.
15. USP <1225>; *Validation of Compendial Methods* (2014)
16. *Guidelines for the validation and verification of quantitative and qualitative test methods*. National Association of Testing Authorities, Australia, (2012).
17. World Health Organization; Supplementary guidelines on good manufacturing practices: validation Annex 4; WHO Technical Report Series, No. 937 (2006).
18. Center for Drug Evaluation and Research (CDER); *Reviewer Guidance 'Validation of Chromatographic Methods'* (1994).
19. Guidance for Industry Analytical Procedures and Methods Validation Chemistry, Manufacturing, and Controls Documentation; U.S. Department of Health and Human Services Food and Drug Administration (2000).
20. IUPAC; Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis; *Pure and Applied Chemistry*, (2002); 74: 835–855.