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Original Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR ISOSULFAN BLUE BY LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: To develop and validate new, simple and rapid analytical method for determination of related impurities in isosulfan blue drug substances by the liquid chromatographic method as per ICH guidelines.

Methods: The chromatographic separation obtained between drug substance i.e. isosulfan blue and its related impurities (Impurity-A, Impurity-B and Impurity-C) on C18 (100 x 2.00 mm) 1.9µ UPLC column using a mobile phase system containing 0.1 % perchloric acid in water (Mobile phase A) and 0.1 % perchloric acid in mixture of 30 volumes of water and 70 volumes of acetonitrile (Mobile Phase B) with gradient program; detector wavelength 220 nm and column temperature 30 °C. The developed method was extensively validated according to ICH guidelines.

Results: Good linearity was observed for isosulfan blue, impurity-A, impurity-B and impurity-C, linearity was calculated from loq Level To 150%with respect to specification level. The correlation coefficient R = 0.999 was proved and showed that the method is robust. The limit of detection of isosulfan blue, impurity-A, impurity-B and impurity-C were found to be 0.010%, 0.015%, 0.030% and 0.0075 % respectively and limit of quantitation of isosulfan blue, impurity-A, impurity-B and impurity-C were found to be 0.030%, 0.030%, 0.045% and 0.015% respectively for 2µl injection volume. The percentage recovery of isosulfan blue and its related impurities were ranged from 94.0 to 108.0 in bulk drug samples. Isosulfan blue sample solution and mobile phase were found to be stable for at least 72 h. The proposed method was found to be suitable and accurate for the quantitative determination of impurity-A, impurity-B, impurity-C and other unknown impurities in isosulfan blue drug substances.

Conclusion: A new, simple and rapid method has been developed and validated for separation and determination of impurity-A, impurity-B, impurity-C and unknown impurities of isosulfan blue by the reverse-phase liquid chromatographic method. Analytical method was developed and validated as per ICH guidelines.

The developed method can be used for the quantitative determination of impurity A, impurity B, impurity C and unknown impurities in isosulfan blue drug substances in pharmaceutical industry.

Keywords: Isosulfan blue, Known impurities, UPLC, Reverse phase, Validation, Solution stability

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INTRODUCTION

Isosulfan blue, having a chemical name, N-[4-[[4-(diethylamino) phenyl](2,5-sulfophenyl) methylene]-2,5-cyclohexadien-1-ylidene]-N-ethylethanaminium,sodium salt [1]. Isosulfan blue is used as a cancer diagnostic agent [1]. Other names are sulfan blue or patent blue is an active pharmaceutical ingredient (API's) [2].

The chemical formula is $C_{27}H_{31}N_2NaO_6S_2$; chemical structures show in (fig. 1). The chemical structure has been obtained from a process for the preparation of isosulfan blue," U. S. Publication number US7534911 [1]. Impurity-A (Des ethyl analogue) is a process related impurity, may arise during the manufacturing process of ISO-IV. During oxidation of ISO-III this impurity formation is possible, the chemical structure shown in (fig. 2). Impurity-B (patent blue) is process related impurity. This impurity is patent blue, which is isomeric impurity of isosulfan blue, chemical structure show in (fig. 3). Impurity-C (ISO-III) is process related impurity. This is carryover impurity of ISO-III and may arise during the manufacturing process of Isosulfan blue, chemical structure show in (fig. 4).

In the literature, there is no analytical method was reported for qualitative and quantitative analysis of Isosulfan blue and its related impurities.

This report describes a reverse-phase ultra-performance liquid chromatography method for the rapid separation of impurity A, impurity B, impurity C (known impurities) and isosulfan blue on by using C18 (100 x 2.00 mm) 1.9µ column. The developed UPLC method was validated for quantification of impurity A, impurity B, impurity C and unknown impurities in isosulfan blue as per validation of analytical procedure guidelines i.e. ICH guideline: Q2(R1) Validation of analytical procedures: Text and methodology [6].

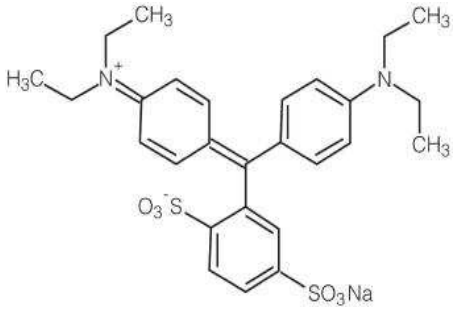


Fig. 1: Chemical structure of isosulfan blue

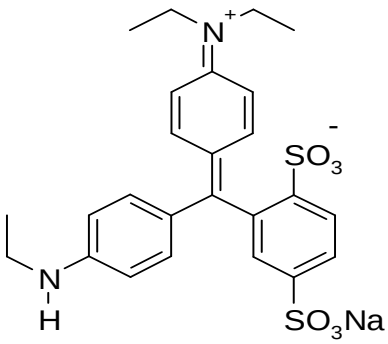


Fig. 2: Chemical structure of impurity-A (Des ethyl analogue)

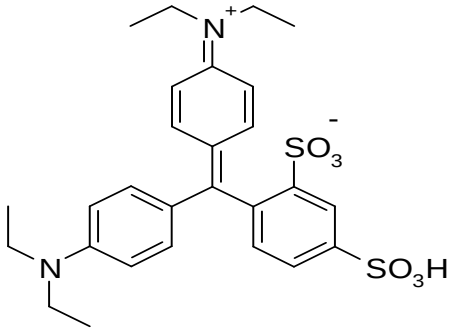


Fig. 3: Chemical structure of impurity-B ((Patent Blue)

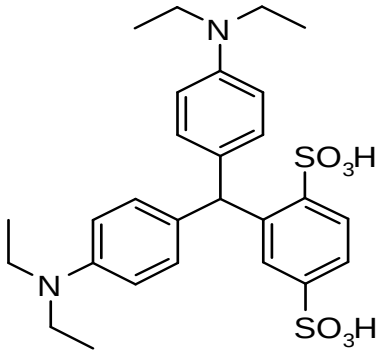


Fig. 4: Chemical structure of impurity-C (ISO-III)

MATERIALS AND METHODS

Chromatographic method is developed and validated as per ICH guideline [6].

Method details are as follows:

Chemicals

Isosulfan blue, impurity A, impurity B and impurity C were kindly gifted by Emcure Pharmaceuticals Ltd Pune, Maharashtra, India. AR grade perchloric acid purchased from JT baker, HPLC grade acetonitrile purchased from biosol, AR grade sodium dihydrogen phosphate dihydrate purchased from Merck and Millipore water is used for mobile phase preparation and diluent preparation.

Equipment

Waters Acquity UPLC system with UV and PDA detector and the inbuilt auto-injector was utilized for method development and validation. Empower2 software was used for data acquisition and system suitability calculations.

Standard preparation

Weigh accurately about 10 mg of isosulfan blue standard and 15 mg of each impurity standard of impurity A, impurity B and impurity C in 100 ml volumetric flask dissolve in diluents and dilute up to the mark (stock solution) further pipette out 10 ml of stock and dilute it to 200 ml. Further pipette 10 ml above solution in 100 ml volumetric flask.

Sample preparation

Weigh accurately and transfer about 25.0 mg of sample in 50 ml volumetric flask, dissolve and dilute to volume with diluents.

Chromatographic conditions (method)

The chromatographic conditions were optimized using a YMC Triart C18 (100 x 2.00 mm) 1.9µ column. The flow rate was set at 0.35 ml/min. The column was maintained at 30 °C and the detection was carried out at a wavelength of 220 nm. The injection volume was 2µl.

Gradient program: Time–mobile phase A,0.0-95, 10.00-30, 15.00-20, 15.50-95, 17.00-95

Mobile Phase

Mobile phase preparation-A

0.1% perchloric acid in water

Mobile phase preparation-B

0.1% perchloric acid in a mixture of 30 volumes of water and 70 volumes of acetonitrile.

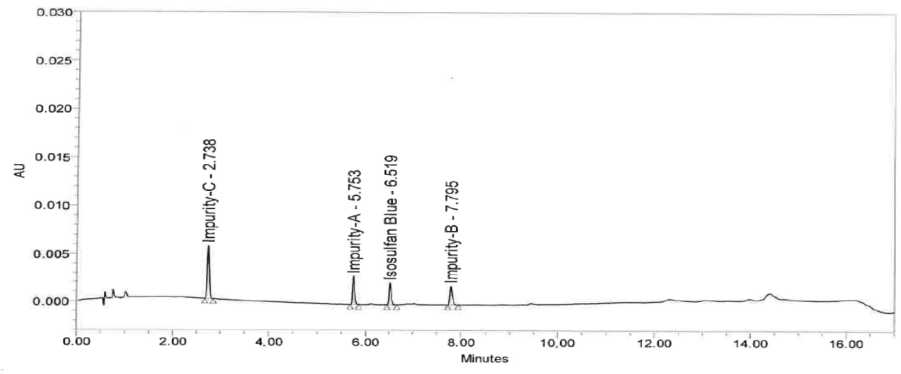
Diluent Preparation

10 mmol of sodium dihydrogen phosphate dihydrate water.

Validation of the method

Specificity

Specificity study was carried out to verify there is no any blank interference with respect to the peak of interest, all the peaks are separated properly and peak purity of all individual peaks are complying typical ultra-performance liquid chromatography chromatogram showed in (fig. 5). The results are tabulated in (table 1).



	Peak Name	RT	Area	% Area	USP Resolution
1	Impurity-C	2.74	15716	41.71	
2	Impurity-A	5.75	8652	22.96	39.24
3	Isosulfan Blue	6.52	6809	18.07	9.54
4	Impurity-B	7.80	6501	17.25	13.98
None					

Fig. 5: Typical ultra-performance liquid chromatography chromatogram with data of system suitability (standard)

Table 1: System-suitability (specificity) report

Compound	Rt	R_S	N	T
Impurity-C	2.74	-	21406	1.18
Impurity-A	5.75	39.24	90910	1.36
Isosulfan Blue	6.52	9.54	100761	1.38
Impurity-B	7.80	13.98	119938	1.48

R_S-USP resolution, N-number of theoretical plates (USP tangent method), T-USP tailing factor

Forced degradation

As part of specificity study, product was subjected for degradation under different conditions like acidic, basic, oxidation, thermal, photolytic, humidity conditions. In Degradation study, it is ensured that all the peaks are separated and complying for peak purity.

Limit of detection and quantitation

The lower concentration levels of analyte i.e. isosulfan blue and its related impurities prepared and injected. The limit of detection, limit of quantitation values have been determined from signal to noise ratio and are tabulated in (table 2).

Table 2: Limit of detection and limit of quantitation

Compound	LOD (%) w. r. t-test	LOQ (%) w. r. t-test
Isosulfan Blue	0.010	0.030
Impurity-A	0.015	0.030
Impurity-B	0.030	0.045
Impurity-C	0.0075	0.015

LOD-Limit of detection, LOQ-Limit of quantitation

Limit of quantitation precision

For the limit of quantitation precision study, six replicate injections of isosulfan blue and known impurities at the limit of quantitation concentration were injected. Percent relative standard deviation for the peak area of isosulfan blue, known impurities and signal to noise ratio was evaluated [6].

Linearity and range

Linearity was calculated from limit of quantitation level to 150 % with respect to specification level for isosulfan blue, impurity A, impurity B and impurity C. The range was selected from limit of quantitation, 50%, 80%, 100%, 120% to 150% of specification limit for impurity A, impurity B, impurity C, and isosulfan blue (For any unspecified unidentified impurity).

Precision

System precision

Blank solutions and six replicate injections of standard solution were injected as per methodology. Percent relative standard deviation of area obtained from six replicate injections of standard solution was calculated. Results are tabulated in (table 3).

Table 3: System precision results

Compound	Relative standard deviation in percent
Isosulfan blue	1.7
Impurity A	0.8
Impurity B	3.1
Impurity C	0.9

Method precision

Method precision was demonstrated by spiking the known impurities at specification level in the sample. One unspiked sample and six different samples of the same batch spiked with known impurities were prepared and analyzed as per methodology. Calculated percent impurity level and relative standard deviation for isosulfan blue and impurities. Results are tabulated in (table 4).

Intermediate precision

The similar procedure of method precision was carried out by the different analyst, on different day, on a different instrument, by different column lot number using the same sample used in method precision as per methodology. Percent relative standard deviation of results was calculated.

Overall percent relative standard deviation of method precision and intermediate precision results was also calculated. The tabulated data shows that the analyst first has done the method precision and analyst second has done the intermediate precision. Results are tabulated in (table 5).

Accuracy (Recovery)

The recovery level solutions were prepared by spiking impurity A, impurity B and impurity C at the limit of quantitation, 50%, 100% and 150% of the specification level in the sample. Two preparations of each level were made and injected as per methodology % Recovery mean recovery and % relative standard deviation calculated. Results are tabulated in (table 6).

Table 4: Method precision results

Method precision: (n=6)		
Compound	**Mean (%)**	**RSD (%)**
Impurity-A	0.1639	0.73
Impurity-B	0.1444	1.65
Impurity-C	0.1563	0.64
Any unspecified unidentified impurity (Highest)	0.0122	NA
Total impurities	0.4768	0.79

RSD-Relative standard deviation, n-Number of injection, NA-Not applicable

Table 5: Intermediate precision results

Intermediate precision: (n=6)		
Compound	**Mean (%)**	**RSD (%)**
Impurity-A	0.1664	0.73
Impurity-B	0.1426	1.00
Impurity-C	0.1579	0.79
Any unspecified unidentified impurity(Highest)	0.0115	NA
Total impurities	0.4784	0.34
Overall relative standard deviation of method precision and intermediate precision results (n=12)		
Compound	**Mean (%)**	**Overall RSD (%)**
Impurity-A	0.1652	1.05
Impurity-B	0.1435	1.46
Impurity-C	0.1571	0.87
Any unspecified unidentified impurity(Highest)	0.0118	NA
Total impurities	0.4776	0.60

RSD-Relative standard deviation, n-Number of injection, NA-Not applicable.

Table 6: Accuracy (Recovery) results

Level/Impurity		**Impurity-A**	**Impurity-B**	**Impurity-C**
LOQ	Mean % Recovery	103.67	107.52	108.38
	RSD (%)	2.40	2.88	4.60
50%	Mean Recovery (%)	96.86	101.96	95.56
	RSD (%)	0.66	3.70	0.28
100%	Mean Recovery (%)	94.53	99.26	95.04
	RSD (%)	0.69	1.90	0.31
150%	Mean Recovery (%)	94.22	96.99	94.72
	RSD (%)	0.98	1.81	0.37

RSD-Relative standard deviation, LOQ-Limit of quantitation

Solution stability

Solution stability was performed at room temperature. Standard preparation, the test sample and spiked test preparation with known impurities at specification level and injected at 0,6,12.18,24,36,48,72 hourly basis.

Robustness

Robustness of the method was verified by deliberately varying the instrumental conditions such as by changing flow rate ± 10% (Flow= 0.385 ml/min and 0.315 ml/min). By changing column oven temperature ± 5 °C (Temperature 35 °C and 25 °C) and by changing column lot number. The system suitability was evaluated in each condition, and the spiked sample was analyzed in triplicate.

RESULTS AND DISCUSSION

An analytical method has been developed and validated as per ICH guideline for drug substance isosulfan blue. All the validation parameters are studied and results found within guideline requirements. The details are as follows:

Specificity

The peak due to isosulfan blue is well resolved from peak due to its known impurities. The blank does not show any peak at the retention time of isosulfan blue and its known impurities. Peak purity angle is less than peak purity threshold which implies that peak purity passes. Hence it is concluded that method is selective.

Forced degradation

Peak due to isosulfan blue and degradation product are well separated. The peak purity of isosulfan blue in degraded and as such sample found complying as per ICH guideline.

Limit of detection and quantitation

Percent relative standard deviation of the limit of quantitation precision was well within acceptance criteria and found. Below 10%. Hence it is concluded that the method can quantify, reported a limit of quantitation precision with precision.

Linearity and range

The straight-line nature of graph and correlation coefficient was well within the acceptance criteria and found more than 0.99. It shows that method has linearity from the limit of quantitation to 150% of specification limit for impurity-A, impurity-B, impurity-C, and isosulfan blue (for any unspecified unidentified impurity).

Precision

Percent relative standard deviation of area obtained from six replicate injections of standard solution is below 5%. Hence the system is precise for determination of related substances in isosulfan blue.

Method precision

Relative standard deviation of % impurity A, % impurity B, % impurity C, % any unspecified unidentified impurity, % total impurities obtained from six different spiked test preparations found below 5%. Hence it is concluded that the method is precise for the determination of related substances in isosulfan blue.

Intermediate precision

Relative standard deviation of % impurity A, % impurity B, % impurity C, % any unspecified unidentified impurity, % total impurities obtained from six different spiked test preparations found below 5%. Overall percent relative standard deviation of method precision and intermediate precision results is below 5% criteria. It shows that there is no effect of random events on the precision of analytical procedure.

Accuracy (Recovery)

The recovery data is well within the acceptance criteria. Recovery obtained for impurity A, impurity B and impurity C between 80% to 120%. It shows that method is accurate for determination of related substances in isosulfan blue

Solution stability

Standard preparation and sample preparation found stable up to 72 h.

Robustness

The system suitability was evaluated in each condition and the spiked sample was analyzed in triplicate. The results are compared with the method precision by calculating overall percent relative standard deviation and found below 5%. The peak due to isosulfan blue is well resolved from peak due to its known impurities.

CONCLUSION

A new, simple and rapid method is developed and validated as per ICH guideline for separation and determination of impurity A, impurity B, impurity C and unknown impurities of isosulfan blue drug substances by UPLC. The method validation was carried out by using YMC Triart C18 (100 x 2.00 mm) 1.9 µ columns. The developed method can be used for the quantitative determination of impurity A, impurity B, impurity C and unknown impurities of isosulfan blue drug substances in pharmaceutical industry.

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CONFLICTS OF INTERESTS

Declare none

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