International Journal of Pharma Sciences
Vol. 8, No. 2 (2018): 1908-1913
Research Article
Open Access

ISSN: 2320-6810

An efficient, commercially viable and safe process for preparation of Isosulfanblue sodium

Chandra Sekhara Rao Nethinti[1,2,*], Sreenivasulu Boju[1], Raghu Babu Korupolu[2], Annapurna Nowduri[2] and Uttam Kumar Ray[1]

[1] *Chemical Research and Development Department, Aurobindo Pharma Ltd, Survey No:71&72, IndrakaranVillage, Sangareddy Mandal, Medak district, Hyderabad -502329,Telangana, India.*
[2] *Department of Engineering Chemistry, A. U. College of Engineering (A), Andhra University, Visakhapatnam – 530 003, Andhra Pradesh, India.*

* Corresponding author: Chandra Sekhara Rao Nethinti, e-mail: chandu_nethinti@yahoo.com

Received: 15 February 2018 Accepted: 25 February 2018 Online: 02 March 2018

ABSTRACT

An efficient, commercially viable and safe synthetic process for the preparation of a diagnostic drug substance, isosulfanblue sodium with a purity of more than 99.8% was demonstrated. This four stage synthetic process has several advantages over the earlier reported procedures and meeting all the regulatory requirements. The present work further describes the formation and control of the possible impurities.

Keywords: Cancer diagnostic, 2-Chloro Benzaldehyde, N,N-Diethyl aniline, Isosulfanblue, Cheaper oxidizing agent.

1. INTRODUCTION

Isosulfanblue sodium **1** is chemically known as *N*-[4-[[4-(diethyl amino) phenyl](2,5-disulfophenyl) methylene]-2,5-cyclohaxadien-1-ylidene]-*N*-ethyl ethanaminium, inner salt, sodium salt. Isosulfanblue is a triaryl methane dye used as a contrast agent for the delineation of lymphatic vessels and in particularly useful in cancer therapy. It is a 2,5- isomer of sulfanblue or patent blue. Isosulfanblue is an active pharmaceutical ingredient used in **lymphazurin®** blue dye. It has been approved in a procedure called "mapping of the sentinel lymph nodes" [1-3].

It is an adjunct to lymphograsphy for visualization of the lymphatic system region of injection. It has been used with increasing frequency in localizing sentinel lymph nodes in breast cancer patients. Isosulfanblue guided surgical removal of cancerous tissue has been on the rise as it is cost effective and safer to use than technetium 99M radioisotope-labeled sulfur colloid [4,5].

Generally preparation of triarylmethane dyes involves condensation of suitably substituted aryl aldehydes with two equivalents of alkyl-aryl amines giving rise to leuco bases or leuco acids followed by oxidation. Although the literature is replete with methods of preparing triarylmethane dyes most of these methods needs a) strong acids, for condensation resulting in leuco bases or leuco acids b) use of hazardous oxidizing agents such as lead oxides, chloranil, iron phthalocyanine and oxone c) purification of crude intermediates. Crude triaryl methane dyestuffs are mainly used for dyeing fabric, cooling paper and printing inks [6]. Herein we report facile and cost effective synthesis of isosulfan blue with commercial applicability.

2. MATERIALS AND METHODS

2.1 Experimental Section:

^{1}H NMR and DEPT spectral data were obtained in dimethyl sulfoxide (DMSO-d6) at 300 MHz spectrometers. The chemical shift values were reported on the δ scale in parts per million (ppm), downfield

from tetra methyl silane (TMS, δ = 0.0) as an internal standard. Spin multiplicities are given as as (singlet), d (doublet), dd (double of doublet), t (triplet), and m (multiplet) as well as brs (broad). Coupling constants (*j*) are given in Hertz. IR spectra were recorded in the solid state as KBr dispersion using a PerkinElmer spectrum one Fourier transform (FT)-IR spectrophotometer. Mass spectrum was recorded using a PerkinElmer PE SCIEX API 2000, equipped with an ESI source used online with an HPLC system after the ultraviolet (UV) detector. HPLC chromatographic purity was determined by using area normalization method. A preparative HPLC system equipped with Shimadzu consists of LC8A VP pumps with premixer assembly and SPD-10A VP spectrophotometric UV-VIS detector. The data was collected and processed using Shimadzu software. A stainless steel column 500mm long, 30mm internal diameter filled with Octadecylsilane (C18) chemically bonded to pours silica particle size of 10µ [Hyper Prep HS C18, 10µ, 500mm x 30mm; Make: Thermo or PURTAS PREP] was employed for loading the sample. An analytical method was developed in isocratic mode separately to resolve the impurity. The mobile phase consisted of 1.5 v/v formic acid, Milli-Q water and acetonitrile in the ratio of 1.5 v/v : 80 : 20. The flow rate was set at ~30 ml/min. Detection was carried out at 220nm. Approximately 80 mg/ml of sample was prepared using sample diluents. The sample diluent is Milli-Q water. The thermal analysis carried out on DSC Q 1000 TA. The thermo gram was recorded from 40 to 320°C. The solvents and reagents were used without purification.

2.2.1 *2-Chloro Benzaldehyde- 5- sulfonic acid (3).*

30% Fuming sulfuric acid (227.64 g, based on sulfur trioxide molecular weight, 1138 ml) was charged and cooled to 15-20^{0}C. 2-chloro benzaldehyde (200 g, 1.4723 moles) was added drop wise to the reaction mass at 15-20^{0}C over period of 60 minutes. The reaction mixture was heated to 70^{0}C and stirred for 3 h, the reaction mixture was poured into crushed ice (2400 g) and stirred. Solid sodium chloride (1000 g) was added as a lot wise to the reaction solution to precipitate a light yellow coloured solid. The solid collected by filtration and washed with diethyl ether to afford 2-chloro benzaldehyde-5-sulfonic acid **3** (crude) 800 g. The crude **3** (800 g) was suspended in methanol (4000 ml) at 25-30^{0}C. The reaction mixture was stirred for 1 h at 50-55^{0}C, inorganic salts were filtered through hyflo bed and the filtrate was evaporated under reduced pressure to obtain solid residue. This residue was treated with methanol (300 ml) at 0-5^{0}C, collected by filtration to give 2-chlorobenzaldehyde-5-sulfonic acid pure **3** (280 g, 89.23%) with chromatography purity 99.76% performed by HPLC. **IR** (KBr, cm^{-1}); 3445, 3064, 2869, 2755.82, 1942, 1695, 1635,1592, 1460, 1368, 1270, 1244, 1195, 1110, 1042, 906, 897., 835, 745, 699, 665; **^{1}H NMR** (DMSO, 500 MHz, δ): 7.60 (d, 1H), 7.86 (d, 1H), 8.07 (s, 1H), 10.33 (s, 1H); **MS** m/z (ESI): 221.5 [(MH)$^+$], 218.9 [(MH)$^-$].

2.2.2 *Benzaldehyde-2,5- disulfonic acid, disodium salt (4).*

2-chlorobenzaldehyde-5-sulfonic acid **3** (200 g, 09070 moles), sodium sulfite (262.86 g, 2.086 moles) and sodium bisulfate (26.4 g, 0.254 moles) were dissolved in water (1600 ml). The solution was charged in to autoclave. The reaction mixture was stirred and heated at 170-180^{0}C with a pressure of 150 Psi for 2 h. After cooling and releasing the pressure, the reaction mixture was poured into methanol (6400 ml) and stirred. The inorganic salts were removed by filtration. The filtrate was concentrated to give benzaldehyde-2,5-disulfonic acid, and disodium salt **4** (crude) (140 g). The crude **4** (140 g) was suspended in *N,N* dimethyl form amide (1190 ml) and stirred for 1 h at 110-115^{0}C. the mixture was filtered through hyflo and the filtrate was precipitated using dichloromethane (3710 ml) to afford benzaldehyde-2,5-disulfonic acid disodium salt pure **4** (44 g) with chromatography purity 99.16% performed by HPLC. **IR** (KBr, cm^{-1}); 3394, 3059, 2896, 2244, 2100, 1713, 1686, 1589, 1467, 1404, 1394, 1297, 1229, 1206, 1128, 1083, 1052, 1022, 906, 840, 755; **^{1}H NMR**(DMSO, 500 MHz, δ): 8.11-8.17 (m, 2H), 8.34 (s, 1H), 10.76(s, 1H); **MS** m/z (ESI): 310.92 [(MH)$^+$].

2.2.3 *N-4-[bis [4-(diethylamino) phenyl] methyl]-benzene-2, 5-disulphonic acid (isoleuco acid) (6).*

Benzaldehyde-2,5-disulfonic acid disodium salt **4** (40 g, 0.1509 moles) and glacial acetic acid (640 ml) were charged into a round bottom flask, *N,N* diethyl aniline **5** (40.4 g, 0.2711 moles) was added to the reaction mixture and refluxed at 40-45^{0}C. The reaction mass was cooled to room temperature. Methanol (400 ml) was added and then solid was separated and collected by filtration and washed with methanol to obtain isoleuco acid **6** (48 g, 68.18%) with chromatographic purity 99.28% performed by HPLC.**IR** (KBr, cm^{-1}); 3445, 3064, 2869, 2755, 1942, 1695, 1635,1592, 1460, 1388, 1270, 1244, 1195, 1110,1042, 906, 897, 835,745, 699; **^{1}H NMR** (DMSO, 500 MHz, δ): 1.12 (t, 12H), 3.64 (m, 8H), 6.89 (s, 1H), 7.36-7.49 (m, 8H), 7.53 (s, 1H), 7.83 (2d, 2H); **MS** m/z (ESI): 547[(MH$^+$)], 545[(MH)$^-$].

2.2.4 *N-[4-[[4-(diethylamino) phenyl] (2, 5-disulfophenyl)-methylene]-2, 5-cyclohexadien-1-ylidene]-n-ethylethanaminium inner salt, sodium salt 1 (crude).*

Isoleuco acid **6** (45 g, 0.0824 moles) was suspended in water. Manganese dioxide (17.91 g, 0.204 moles) was added to the stirred suspension in one portion at room temperature. 50% aqueous phosphoric acid was added to lower pH to 2.2 at room temperature and pH was adjusted at every one hour to 2.2 to check the absence of isoleuco acid. If isoleuco acid is absent and the salts were filtered and washed with water. The pH of the filtrate was adjusted to 7.0-7.5 with 8% aqueous sodium bi carbonate solution and filtered through 0.42mµ filter paper. The compound was purified through preparative column chromatography to yield isosulfanblue **1** (crude) (31.5 g, 70%) with chromatography purity 99.86% performed by HPLC.**IR** (KBr , cm^{-1}); 3855, 3839, 3819, 3799, 3748, 3736, 3435, 2976, 2930, 2870, 1970, 1619, 1578, 1499, 1479,

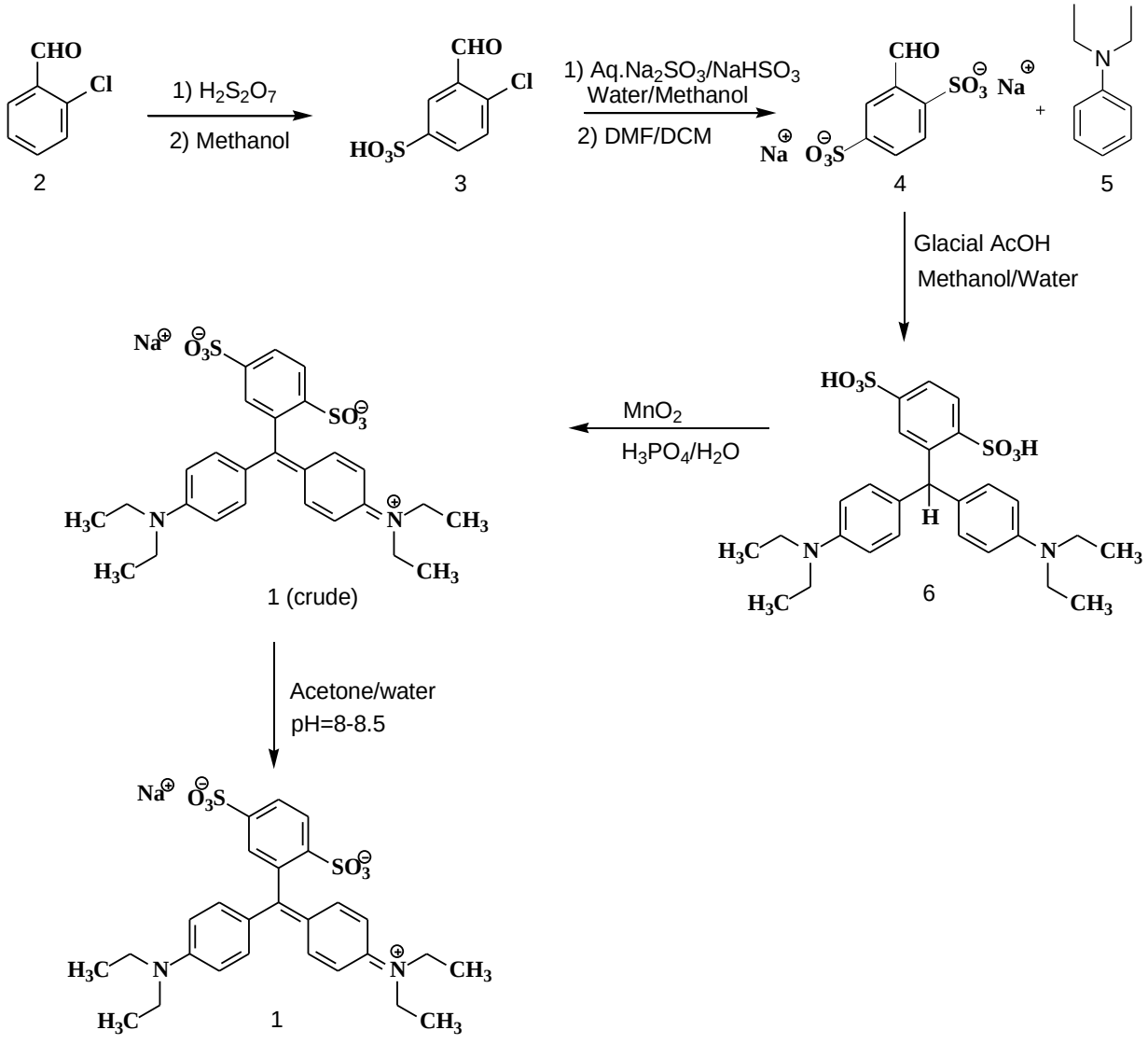
1415, 1391, 1340, 1279, 1222, 1184, 1156, 1120, 1068, 1036, 1016, 1005, 922, 892, 841, 796, 758, 749, 740, 704, 687, 660. **^{1}H NMR**(D_2O, 500 MHz, δ): 1.20 (t, 12H), 3.57 (q, 8H), 6.85 (d, 4H), 7.28 (d, 4H), 7.62 (d, 1H), 8.10 (dd, 1H) , 8.18 (d, 1H); **MS** m/z (ESI): 545[$(MH)^+$], 543.12[$(MH)^-$]. Sodium content is 2.1% w/w.

2.2.5 *N-[4-[[4-(diethylamino)phenyl](2,5-disulfophenyl)-methylene]-2,5-cyclohexadien-1-ylidene]-n-ethylethanaminium inner salt, sodium salt (Isosulfan blue) (1).*

Isosulfan blue **1** (crude) (30 g, 0.055 moles) was dissolved in water. Aqueous sodium bi carbonate solution was added drop wise to the reaction mixture to adjust pH to 8.0-8.5 and continued stirring at 20-25^{0}C for 1 hour. Acetone (1260 ml) was added to the reaction mixture and stirred at 20-25^{0}C for 2 hours. The crystallized product was filtered and the solid obtained was dried at 40^{0}C to yield isosulfanblue sodium **1** (25.5 g, 82.52%) with chromatographic purity 99.91% performed by HPLC. **IR** (KBr, cm^{-1}); 3434, 2975, 2930, 2871, 2625, 1927, 1619, 1578, 1414, 1391, 1340, 1279, 1222, 1184, 1156, 1119, 1068, 1036, 1016, 1004, 921, 892, 863, 840, 833, 796, 758, 740, 740, 687, 659. **^{1}H NMR** (D_2O, 300 MHz, δ): 1.20 (t, 12H), 3.57 (q, 8H), 6.85 (d, 4H), 7.28 (d, 4H), 7.62 (d, 1H), 8.10 (dd, 1H), 8.18 (d, 1H); **MS m/z** (ESI): 545[$(MH)^+$], 543.12[$(MH)^-$]. Sodium content is 4.3% w/w and manganese content is < 3.0ppm.

3. RESULTS AND DICUSSION

Our synthesis [7] (Scheme 1) commenced from the commercially available 2-chloro benzaldehyde **(2)** and *N,N*-diethyl aniline **(5)**. The process comprises of reacting compound **2** with a sulfonating agent (30% fuming sulfuric acid) the reaction was exothermic and the temperature raise up to 70^{0}C. Later the reaction mixture was poured into crushed ice followed by the addition of solid sodium chloride in portions to the acidic solution to precipitate 2-chloro benzaldehyde 5-sulfonic acid **3** which was isolated by filtration.



Scheme 1: synthetic approach towards the preparation of 1

The above product contains inorganic salts and due to their presence, further reactions are not moving properly. Hence to make the reaction productive, we have opted the purification of compound **3**. Compound **3** was purified by suspending in methanol and heated to 50-55^{0}C. The inorganic salts were filtered through hyflo and the filtrate was evaporated under reduced pressure. The pure compound **3** was isolated by conventional techniques such as filtration and drying.

The process comprises, reacting pure compound **3** with sodium sulfite and sodium bi sulfite under closed vessel at 170-180^{0}C in water. After completion of reaction, the contents were cooled to 25^{0}C and thereafter quenched with methanol. The precipitated inorganic salts were filtered and the filtrate was concentrated to produce benzaldehyde-2,5-disulfonicacid,disodiumsalt **4** as a crude product. Compound **4** which contains the dissolved inorganic salts was further, purified by suspending in *N,N*- dimethylformamide and at 110-115^{0}C, followed by filtration through hyflo and the filtrate is precipitated using a solvent from dichloromethane to produce pure benzaldehyde-2,5-disulfonic acid, disodium salt **4** [8, 9].

The pure compound **4** was condensed with *N, N*-diethyl aniline **5** in presence of glacial acetic acid, followed by addition of methanol to produce isoleucoacid **6** [10]. Oxidation of compound **6** was carried out with an oxidizing agent manganese dioxide to produce isosulfanblue **1** (crude).

There are many reports described in the literature for oxidation with different oxidizing agents [11-15]. The major disadvantage with the process are that the intermediate **3**, which is not pure and contaminated with inorganic salts and is carried forward, as such in the finished product **1**. Removal of these impurities in the final stage is very difficult and requires repeated crystallizations. This results in the low yield of compound **1**, which is not preferable for industrial scale-up operations as well as economical point of view. Another major disadvantage with this process is during oxidation unwanted desethyl impurity **7** is generated along with compound **1** (crude)**,** which is very difficult to remove through the crystallization methods [16,17].

In our present invention we also developed process for the purification of compound **1** (crude) by preparative HPLC. Compound **1** (crude) is converted to its sodium salt by treating with a sodium ion source, followed by crystallization to produce highly pure isosulfanblue sodium **1** with a chromatography purity < 99.9%.

Ravishankar *et al.,* [11] have reported yet another process, where the oxidation was carried out in methanol using silver oxide over a period of 12-14 h at 25-30^{0}C. After completion of reaction, the reaction mixture was filtered through buchner funnel to remove solid silver oxide and the filtrate was washed several times with methanol. Further, the combined methanolic filtrate was passed through silica gel or celite and then through an acidic zeolite bed followed by micron filtration. The above mentioned process is very costly, laborious and also fails the test for heavy metals. To overcome the above drawbacks, we have studied the reaction with various oxidizing agent's like manganese dioxide, potassium dichromate, DDQ etc. and screened with different solvents like water, toluene, methanol and THF as shown in Table1. From our experimental studies, we have observed that manganese dioxide is so cheaper compared with reported process, and attaining good yields [18, 19]. Further, the reaction was carried out in a cheaper solvent as water with lesser time makes this process feasible for commercialization in large scale (Table 1; Entry 6).

Table1: Screening of different oxidizing agents for oxidation.

Entry	Oxidizing Agent	Solvent	Mol ratio	Time (h)	Purity by HPLC(%)[a]
1	Cumene, Hydrogen peroxide	Methanol	4	20	0.61
2	$K_2Cr_2O_7$	Methanol	5	10	2.10
3	$K_2Cr_2O_7$ / 40% H_2SO_4	Methanol	5	12	5.20
4	DDQ	Toluene	15	24	ND
5	Desmartin	THF	5	10	ND
6	MnO_2	Water	2.5	4	94.00

[a]reaction monitoring condition; ND- Not detected

After selecting the oxidizing agent and solvent for the oxidation we evaluated mole ratio of manganese dioxide (Table 2). On the basis of the below results, it was observed that manganese dioxide mole ratio is fixed as 2.5 m.eq (Table 2; Entry 5).

Table 2: Screening of MnO_2 with different mole ratio at various temperatures.

Entry	Oxidizing Agent	Mol ratio	Solvent	Temperature (°c)	Time (h)	Purity by HPLC(%)[a]	Isolated Yield (%)
1	MnO_2	25	Toluene	110	12	45.67	32
2	MnO_2	2	Water	95	2	40.46	28
3	MnO_2	3	Water	95	0.5	53.48	37
4	MnO_2	3	Water	27	0.5	71.1	49
5	MnO_2	2.5	Water	27	4	93.2	70

[a]reaction monitoring condition

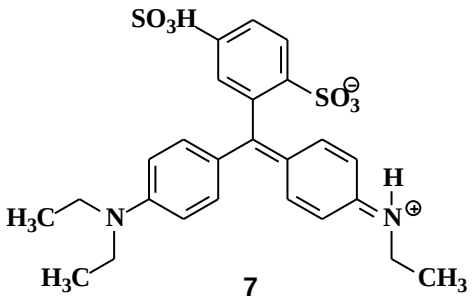
In oxidation another key parameter, the pH was studied. On the basis of experimental studies it was concluded that the pH 2.2-2.6 was the ideal pH for oxidation (Table 3).

Table 3: Screening of pH and Temperature.

Entry	pH	Temperature (°c)	Purity by HPLC (%)[a]	Time (h)
1	2.0	95	40.46	2
2	2.0-2.2	27	73.29	3
3	2.5-3.0	10	13.50	3
4	1.0-1.5	27	65.00	0.5
5	3.5-4.0	27	36.20	5
6	7.0	80	7.80	2
7	2.2-2.6	27	94.00	4

[a] product conversion during pH adjustment

The reaction maintained at pH 7.0 with a temperature of 80^0C, improper reaction was observed without any impurity formation (Table 3; Entry 6). If pH is adjusted for 2.0 initially and then temperature raised to 95^0C we get only 40.46% product purity along with desethyl isosulfanblue **7** (Table 3; Entry 1), which is very difficult to remove through any purification techniques. This results in the low yield of compound **1** (crude). This problem was overcome by maintaining the pH to 2.2-2.6 till the absence of compound **6** (Table 3; Entry 7).



7

Figure 1: structure of impurity 7

If pH was brought down below 2.2 there was a formation of compound **7** with the product there by losing the yield of compound **1** (crude).

After oxidation of compound **6**, the reaction mixture was filtered to remove the inorganic salts and the pH of the filtrate was adjusted to 7.0-7.5. Further, the obtained salts were filtered. From the filtrate, compound **1** (crude) was isolated by preparative column chromatography with >99.8% HPLC purity. This crude was dissolved in water and adjusted to pH 8.0-8.5 by adding sodium bicarbonate. Thereafter it was diluted with acetone to obtained compound **1** with good yield (88.4% of theoretical) and a purity of < 99.9%.

Finally the redesigned process furnished compound **1** with an overall yield of 20.12% from compound **2** after five steps with 99.91% purity and meeting other quality parameters (Table 5). We have also determined the sodium content in compound **1** by ICP-OES to ~ 4.2-4.5 % w/w.

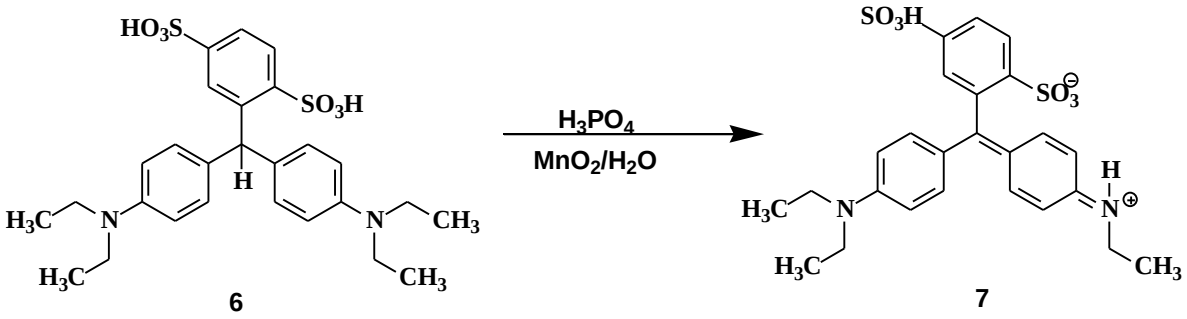
Table 4: Analysis of compound 1.

Entry	Purity by HPLC (%)		Residual Solvents by GC (ppm)						Overall Yield (%)
	1	7	Acetone	ACN	MeOH	DCM	AcOH	DMF	
1	99.91	0.07	451	ND	58	ND	ND	325	20.17
2	99.90	0.08	491	ND	53	ND	ND	301	20.10
3	99.91	0.07	482	ND	51	ND	ND	312	20.12

ND- Not detected

To check the analytical parameters, we have synthesized the impurity **7** by treating compound **6** with manganese dioxide and phosphorous acid in water at 25^0C, maintaining the pH ~1. The enriched impurity

was finally isolated through column chromatography and characterized by ^{1}H NMR, ^{13}C NMR and mass spectrometry.



Scheme 2: Synthesis of Desethyl impurity 7

4. CONCLUSION

An efficient, commercially viable, and safe process has been developed for the synthesis of compound **1** with an overall yield of 20.1 w/w and ~99.9% purity. The drug substance **1**, synthesized through this protocol, complies with all the regulatory requirements. The process described in this article is more advantageous than the reported procedures, due the usage of manganese dioxide for oxidation which eventually reduced the overall cost of the process.

5. ACKNOWLEDGEMENTS

We thank Aurobindo pharma limited for supporting this research work. We extend out thanks to colleagues of Analytical research department and Chemical research department of APL research centre for their cooperation.

6. REFERENCES

1. Lesile LM, Alisa CT, Kimberly JV, Jane F, Alexandra SH, Mary G; Elisa P, Jeanne P, Hiram SC, and Patrick IB, isosulfanblue dye reactions during sentinel lymph node mapping for breast cancer. Anesth Analg 2002; 95:385-8.
2. Armando EG, Daniel MK, J. MG, Donald LM. lymphatic mapping and sentinel lymphadenectomes for breast cancer. ANNALS of SURGERY Vol.220. No.3. 391-401.
3. Paul JR, Jamie T, Jerry IH, and John C. intraoperative identification of chylous fistulas using isosulfanblue dye. AJR 139;186-187, July 1982.
4. Richard T, Todd AA, Jessica LC, David JH, William CE, David RV, Roger YT. bioconjugate chem. 2010; 21:1811-1819.
5. Dave SBH, Bret T. molecular lymphatic mapping of sentinel lymph nodes. US 7,897,336 B2, 2011.
6. V. See, Rodd's chemistry of carbon compounds by S. Coffey, 1974 2nd edition, Volume III part F, 110-133.
7. Aminul I, Uttam KR, Boju S, Meenakshisunderam S. An improved process for the preparation of isosulfanblue. 3509/CHE/2012 A.
8. Benjamin R. Manufacture of banzaldehyde-2, 5- disulfonic acid US 1,531,507, 1922.
9. Hans JM. Process for the preparation of alkali metal and alkaline earth salts of banzaldehyde-2, 5- disulfonic acid. US 4,710,322, 1987.
10. Walter JB, Otto S. Process for preparing leucotriarylmethane compounds. US 2,726,252, 1955.
11. Ravishankar KM, Satyam NB, Peter XT. Process for preparation of isosulfanblue. US 7,662,992B2, 2010.
12. Balkrishna KK, Sankar C, Girish MK, Hawaldar TM, Ashok V, Sandeep SS, Somit K. process for the preparation of isosulfanblue. US 7,534,911 B2, 2009.
13. Karl SL. Process for the Oxidation of leuco compounds of the triaryl methane. US 1,805,925, 1931.
14. Thomas G, Udo M. Preparation of di or tri aryl methane by oxidation. US 5,659,053, 1997.
15. Karl HH. Preparation of cationic triaryl methane dyestuffs. US 4,330,476, 1982.
16. Ching CC, Chung SL, FU DM, Cheyan SWJ. Hazardous. Materials. B137, 2006; 1600-1607.
17. Chun CC, Wei Z, Jingyi L, Jincai Z. Environ. Sci. Technol. 2002; 36: 3604-3611.
18. Sabir M, Philip K. Active MnO_2. Oxidative dehydrogenations synthetic communications, 1982; 12(8):637-645.
19. Ila B, George MV. Oxidation with metal oxides-II Tetrahedron Vol. 24. Pp. 1293-1298.

*****