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SYNTHESIS AND SPECTRAL CHARACTERIZATION OF RELATED SUBSTANCES OF ISOPROTERENOL HYDROCHLORIDE, A POTENT NONSELECTIVE BETA-ADRENERGIC AGONIST DRUG

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ABSTRACT

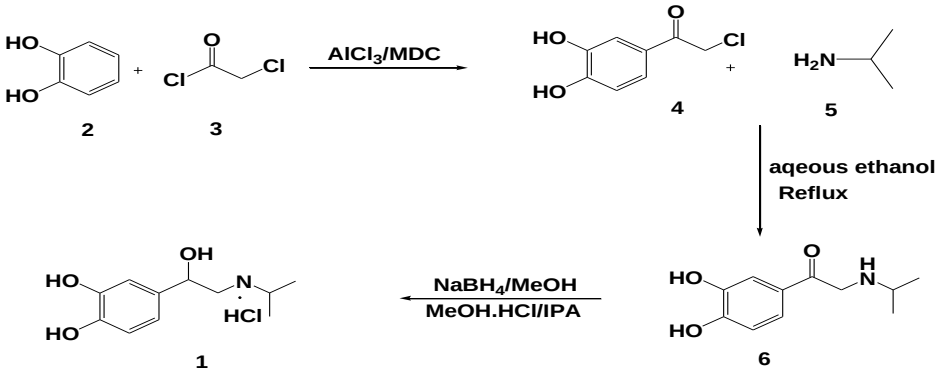
Isoproterenol hydrochloride **1** is used for the treatment of mild or transient episodes of heart block, bronchodilator and Adams-Stokes attack. During the process development of isoproterenol hydrochloride, two process related impurities, 4-[1-methoxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol hydrochloride (**7**) and 4-[1-isopropoxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol hydrochloride (**8**) were identified and these are not reported anywhere and reported herein for the first time. The present work describes the identification, synthesis and spectral characterization of these impurities.

***KEYWORDS*:** Impurities; isoproterenol hydrochloride; adams-stokes attack; bronchodilator; spectral characterization; synthesis.

INTRODUCTION

Isoproterenol hydrochloride (**1**) is a potent nonselective beta-adrenergic agonist used for the treatment of mild transient episodes of heart block, bronchodilator and Adams-Stokes attack. This product is currently being marketed under the name of ISUPREL. It is a racemic compound and structurally related to epinephrine but acts almost exclusively on beta receptors. It is chemically known as (1RS)-1-(3,4-dihyroxy phenyl)-2-[(1-methyl ethyl)amine ethanol hydrochloride]. Isoproterenol hydrochloride is available as an injectable and is administered by the intravenous, intramuscular, subcutaneous or intracardiac routes.[1-4]

The literature survey revealed various synthetic methods for isoproterenol hydrochloride.[5-7]It was synthesized according to the Scheme 1 which is very cheap and commercially viable. Reaction of catechol **2** with chloroacetyl chloride **3** in the presence of lewis acid aluminium chloride gave **4**, which on treatment with aqueous isopropylamine **5** gave **6.** Reduction of **6** with sodium borohydride in methanol gave the title compound isoproterenol hydrochloride **1**. Finally, isoproterenol hydrochloride was purified using aqueous isopropyl alcohol.



Scheme 1: Synthetic method for preparation of isoproterenol hydrochloride (1)

During the process development of isoproterenol hydrochloride, high-performance liquid chromatography (HPLC) analysis of isoproterenol hydrochloride crude product revealed the formation of two major impurities. The presence of these impurities in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug product. As per the guidelines recommended by international conference on harmonisation (ICH),[8,9] the acceptable level for a known and unknown related substances is less than 0.15% and 0.10% respectively. In order to meet the stringent regulatory requirements, the impurities present in the drug substance must be identified. These impurities are required in pure form to understand the complete impurity profile and to check the analytical performance characterization. The present articles describes the identification, synthesis and characterization of these two impurities and root cause of their formation and finally control the formation of these impurities.

MATERIALS AND METHODS

Experimental section

^{1}H and ^{13}C NMR spectral data were obtained in deuterated water(D_2O) at 500MHz and 75MHz spectrometers respectively on Agilent Technologies (Variant). The chemical shift values were reported on the δ scale in parts per million (ppm), downfield from tetramethylsilane (TMS, δ=0.0) as an internal standard. Spin multiplicities are given as a s (singlet), d (doublet), t (triplet) and m (multiplet). IR spectra were recorded in the solid state as KBr dispersion using a Perkin-Elmer Spectrum One Fourier Transform (FT) IR spectrophotometer. Mass spectrum was recorded using a Applied Biosystems; MDS SCIEX; API 3000 and LC/MSD SL Agilent Technologies. HPLC measurements were run on Purospher STAR RP-18e, (250 mm x 4.6 mm,5 µm; Make: Merck) with a flow rate of 0.7 mL/min having a column temperature of 40 ^{o}C. UV detection occured at ʎ=280 nm. The solvents and reagents were used without purification.

4-[1-methoxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol hydrochloride (7)

Compound **6** (10 g, 0.00024 mmol) was suspended in methanol (100 mL) and the temperature of the mixture was cooled to 0 ^{o}C. Sodium borohydride (4.52 g, 0.0006 mmol) was added to the reaction mass at 0^{o}C slowly and stirred for 1 h. The pH of the reaction mass was adjusted to 0.5-1.0 with methanolic hydrochloride (Assay ~20%w/w) (152.8 g, 0.0008 mmol) and stirred for 1 h. The precipitated salts were filtered and the filtrate was concentrated completely under reduced pressure at 50 ^{o}C to afford white coloured compound **7**. Yield 7.6 g (0.76%); HPLC: 98.14%; mp: 180-181 ^{o}C; ^{1}H NMR (500 MHz, D_2O) δ 1.35 (d, 6H), 3.22-3.49 (d, 5H), 3.49 (t, 1H), 4.47 (m, 1H), 6.88 (d, 1H), 6.94 (d, 1H), 6.98 (s, 1H); ^{13}C NMR (75 MHz, D_2O) δ 17.88, 18.29, 49.38, 50.91, 56.03, 78.36, 114.43, 116.30, 119.65, 129.07, 144.33, 144.60; IR (KBr, cm^{-1}) 3338, 3040, 2984, 2897, 2499, 1612, 1601, 1514, 1465, 1449, 1430, 1397, 1376, 1350, 1314, 1063, 1006; HRMS (ESI): *m/z* 225; found 226.1454 $[M+H]^+$, Analytical Calculated for 226.1443.

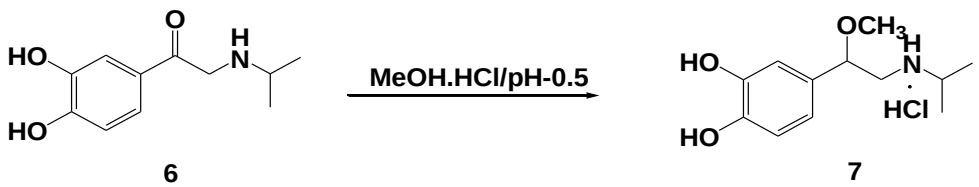
4-[1-isopropoxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediolhydrochloride (8)

Compound **1**(10 g, 0.00004 mmol) was suspended in isopropyl alcohol (50 mL) and the pH was adjusted to 1-1.5 using hydrochloric acid and refluxed for 1 h. Reaction mass was cooled to 0-5 ^{o}C and stirred for 1 h. Filtered the compound and washed with precooled isopropyl alcohol and dried to afford white coloured compound **8**. Yield 5.5 g (0.55%); HPLC: 94.4%; mp: 183-185 ^{o}C; ^{1}H NMR (500 MHz, D_2O) δ 1.09 (d, 3H), 1.19 (d, 3H), 1.41 (d, 6H), 3.18 (d, 2H), 3.51 (q, 1H), 3.67 (q, 1H), 4.72 (t, 1H), 6.88 (s, 1H), 6.97 (d, 2H); ^{13}C NMR (75 MHz, D_2O) δ 17.83, 18.31, 20.30. 20.37, 49.40, 50.86, 51.04, 70.74, 73.85, 114.40, 116.25, 118.55, 130.34, 144.26, 144.41; IR (KBr, cm^{-1}) 3229, 2972, 2809, 2476, 2439, 1606, 1516, 1466, 1428, 1376, 1355, 1286, 1174, 1154, 1133, 1086, 1054, 1017; HRMS (ESI): *m/z* 253; found 254.1773 $[M+H]^+$, Analytical Calculated for 254.1756.

RESULTS AND DISCUSSION

As per ICH guidelines, it is mandatory to identify the possible impurities. During the process development of isoproterenol hydrochloride (Scheme 1), two process related potential impurities were detected in HPLC. To identify the molecular weight of the respective impurities, Liquid Chromatographic-Mass Spectrometry (LC-MS) was performed.

We have prepared two possible impurities and they were characterized by ^{1}H NMR, ^{13}C NMR, Mass, IR and HPLC. These impurities were synthesized and subsequently subjected for spectral analysis [^{1}H NMR, ^{13}C NMR, Mass and IR]. Based on the spectral data, these impurities were characterized as 4-[1-methoxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol hydrochloride (7) and 4-[1-isopropoxy-2-[(1-methylethyl) amino]ethyl]-1,2-benzenediol hydrochloride (8).

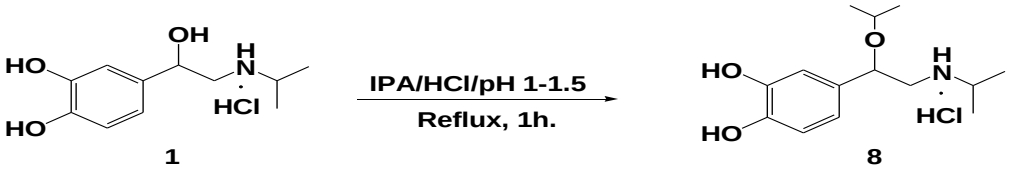


6 **7**

Scheme 2: Synthesis of impurity 7

The compound **7** was synthesized by reduction of **6** with sodium borohydride. After completion of the reaction the mass is quenched with methanolic hydrochloride to pH 0.5-1.0 (Scheme 2). The mass spectrum of **7** displayed a protonated molecular ion peak at *m/z* 225, which is 14 amu greater than **1**. In the ^{1}H NMR spectrum, a singlet signal at 3.2 ppm corresponding to methoxy group with 3 protons integration was observed. Based on these spectral data, the structure of **7** was confirmed as 4-[1-methoxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol hydrochloride. In HPLC it is displayed at 1.34 RRT.

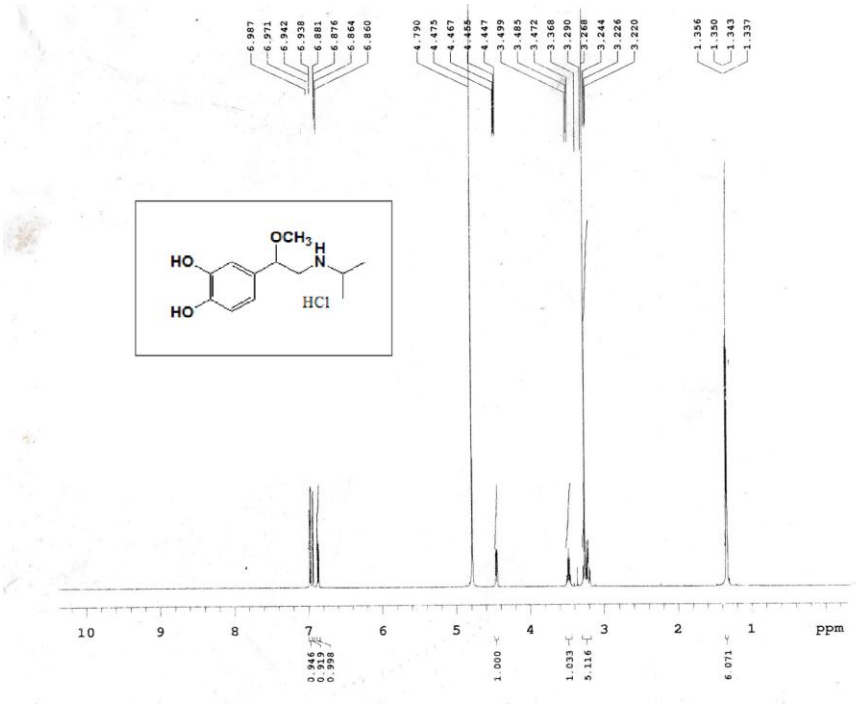
Compound **8** was synthesized by treating **1** with isopropyl alcohol in acidic condition (pH <0.5) (Scheme 3). The mass spectrum of **8** displayed a protonated molecular ion peak at *m/z* 254, which is 43 amu greater than **1**. In the ^{1}H NMR spectrum a doublet at 1.08 and 1.17 ppm was observed corresponding to 2 methyl groups (6 protons) and singlet at 3.6 ppm corresponding O-CH were observed. Based on these spectral data, the structure of **8** was confirmed as 4-[1-isopropoxy-2-[(1-methylethyl)amino]ethyl]-1,2-benzenediol hydrochloride. In HPLC it is displayed at 2.57 RRT.



1 **8**

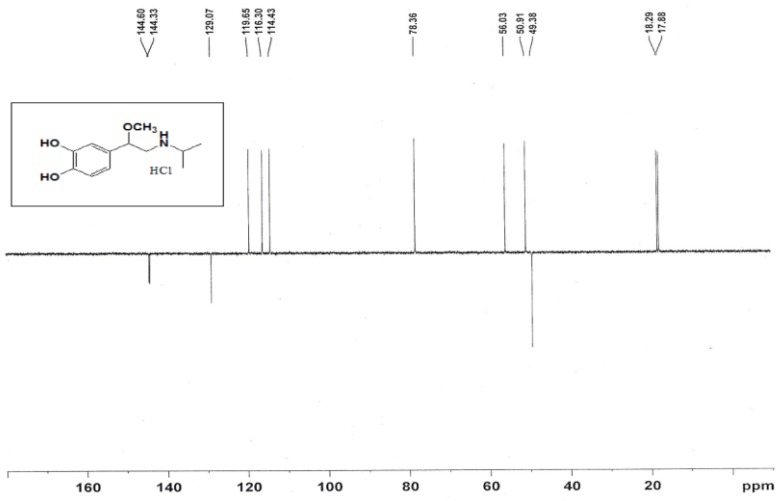
Scheme 3: Synthesis of impuity 8

Compound	**Pages**
1. Characterization of compound **7**(^{1}H NMR, ^{13}C NMR, IR, HRMS, Elemental analysis, HPLC spectrum)	S1-S4
2. Characterization of compound **8** (^{1}H NMR, ^{13}C spectrometry, IR, HRMS, Elemental analysis, HPLC Spectrum)	S6-S9
3. Characterization of compound **1** (^{1}H NMR, ^{13}C spectrometry, IR, HRMS, HPLC Spectrum)	S10-S14

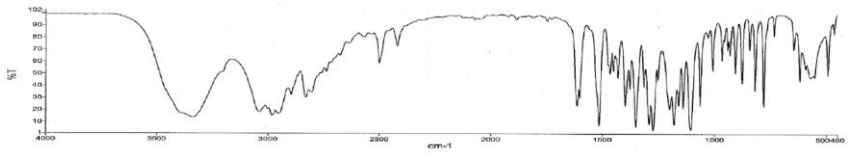


^{1}H NMR Spectrum of Compound 7

S1



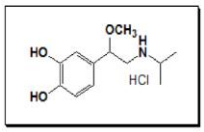
^{13}C NMR Spectrum of Compound 7
S2



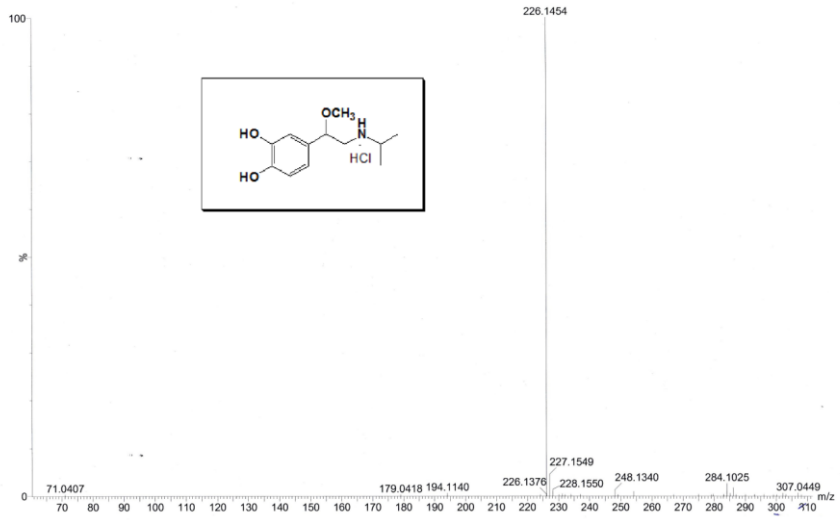
Peak Table

Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)
1	3338.78	13.4755	2	3040.91	18.2322	3	2984.21	15.0899
4	2956.92	16.5717	5	2897.04	32.6091	6	2831.82	30.4298
7	2499.99	59.2898	8	2419.19	73.7055	9	1612.13	23.7525
10	1601.39	30.3914	11	1514.05	7.419	12	1465.11	50.2243
13	1449.78	62.5342	14	1430.02	46.9506	15	1397.52	23.5163

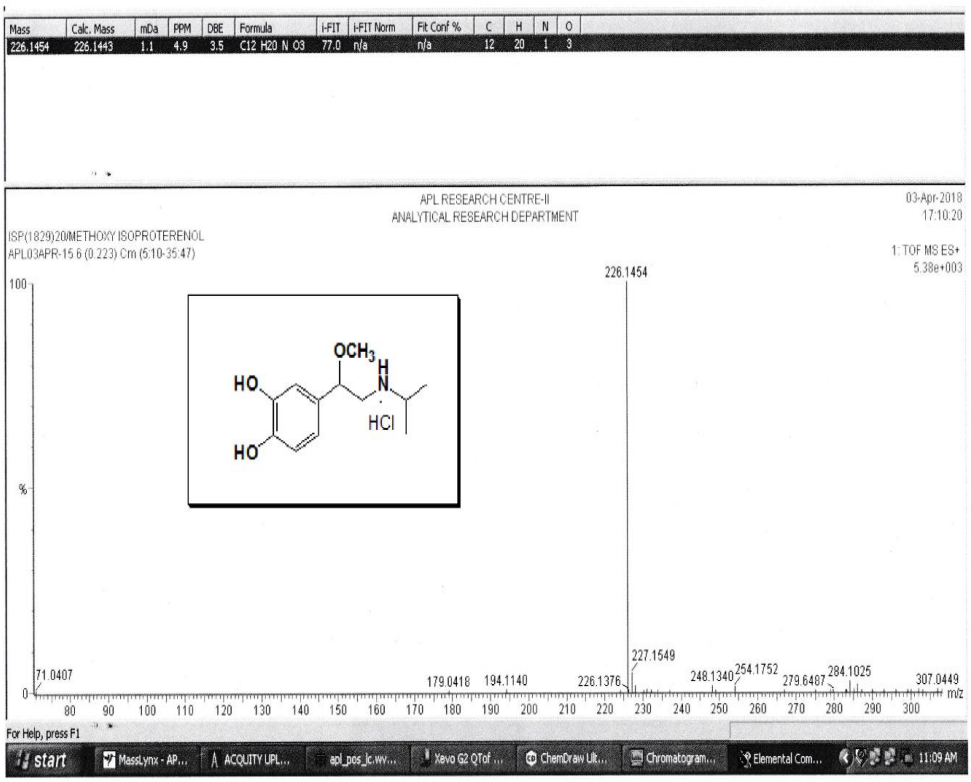
Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)
16	1376.99	37.7014	17	1350.49	6.1445	18	1314.22	39.6591
19	1291.39	8.5457	20	1273.79	2.9966	21	1250.78	44.6419
22	1199.13	20.8521	23	1179.37	8.1013	24	1159.25	23.7473
25	1138.99	21.3509	26	1103.86	3.81	27	1063.21	23.7455
28	1006.78	53.133	29	965.79	61.6827	30	939.01	70.2929
31	929.58	65.7287	32	905.52	50.6423	33	875.2	42.7243
34	840.68	70.6112	35	817.04	36.4801	36	778.92	23.2067
37	732.68	82.2024	38	644.78	70.4892	39	617.31	44.3522
40	591.88	54.2175	41	566.17	47.1603	42	491.36	49.1437
43	464	83.7004						



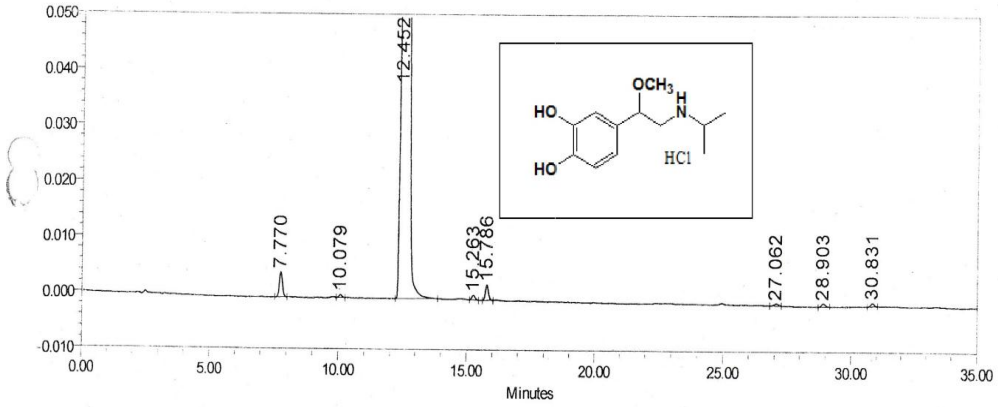
IR Spectrum of Compound 7



Mass Spectrum of Compound 7
S3



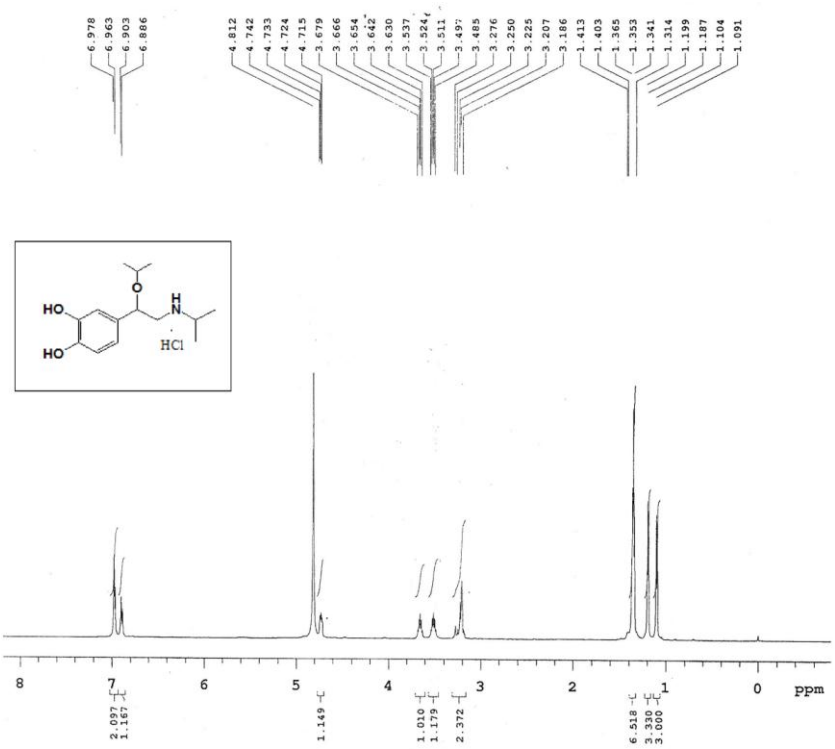
Elemental analysis spectrum of Compound 7



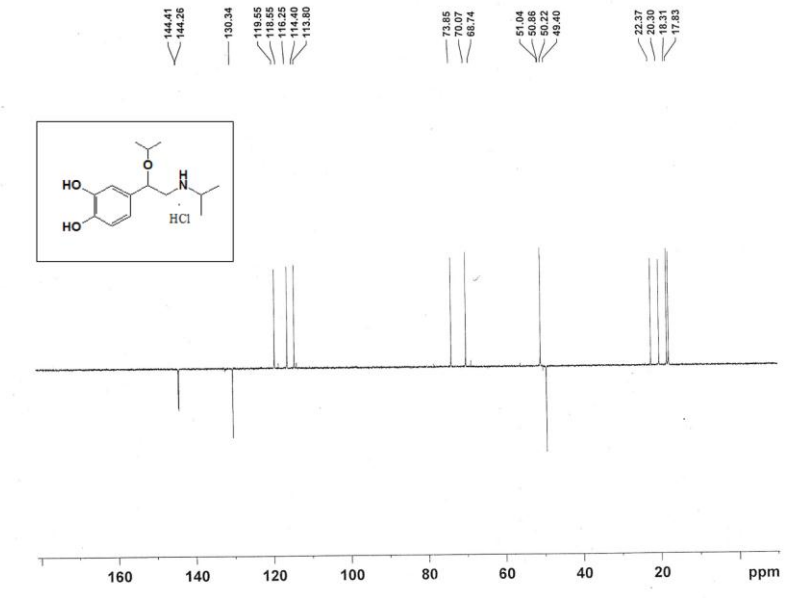
PEAK RESULTS

	RT	Area (µV*sec)	% Area	RT Ratio	Name
1	7.77	36117	0.77	0.62	Peak6
2	10.08	4126	0.09	0.81	Peak7
3	12.45	4578886	98.14	1.00	O-Methoxy Isoproterenol
4	15.26	7514	0.16	1.23	Peak9
5	15.79	22816	0.49	1.27	Peak10
6	27.06	4733	0.10	2.17	Peak11
7	28.90	5882	0.13	2.32	Peak12
8	30.83	5801	0.12	2.48	Peak13

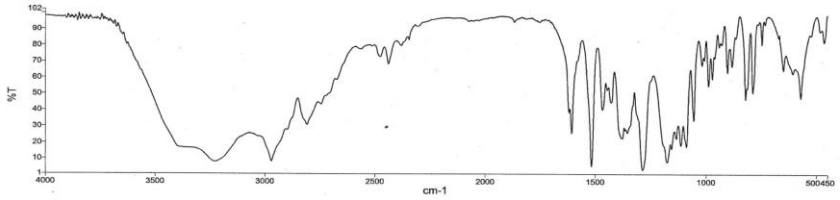
HPLC Spectrum of Compound 7
S4



^{1}H NMR Spectrum of Compound 8
S5



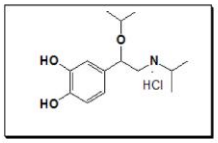
^{13}C NMR Spectrum of Compound 8
S6



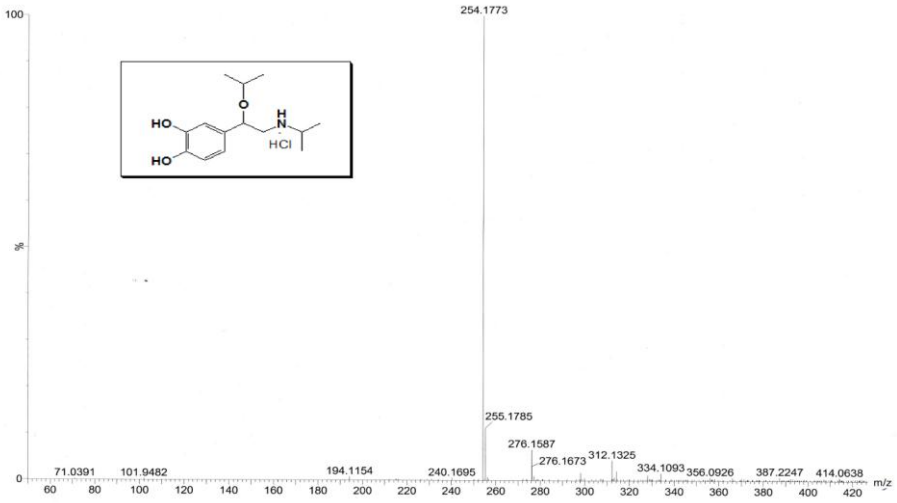
Peak Table

Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)
1	3229.13	7.8854	2	2972.2	8.0656	3	2809.61	30.9089
4	2476.08	73.1046	5	2439.01	68.679	6	1606.8	25.8159
7	1516.24	5.1749	8	1466.63	40.3614	9	1428.84	44.9028
10	1376.47	22.7276	11	1355.03	26.0389	12	1286.3	2.9992
13	1174.02	7.7765	14	1154.15	16.0985	15	1133.22	22.5093

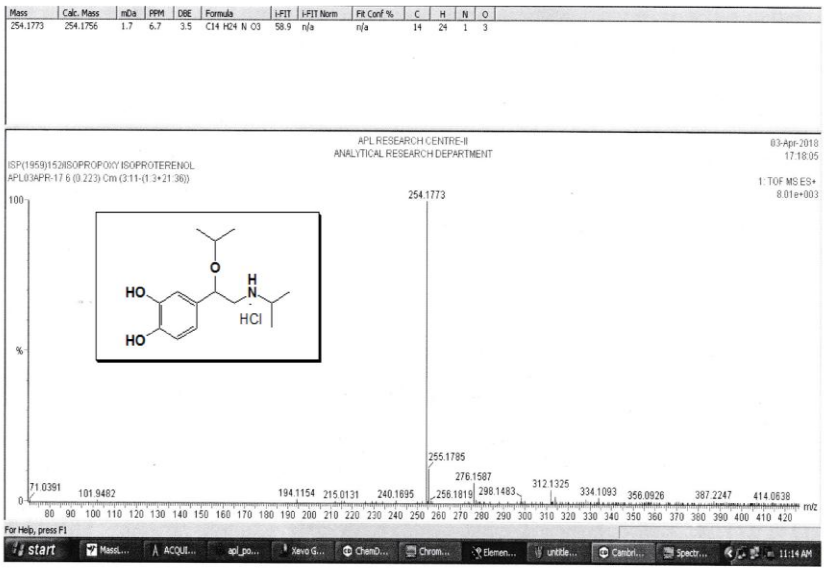
Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)
16	1113	18.2242	17	1086.76	17.3946	18	1054.02	33.712
19	1017.01	67.9875	20	988.09	55.282	21	970.96	59.5522
22	938.92	79.4759	23	901.63	63.5556	24	880.9	67.3875
25	819.84	46.9251	26	787.3	50.8453	27	746.16	81.0835
28	649.74	64.9086	29	569.95	47.677	30	463.94	82.5393



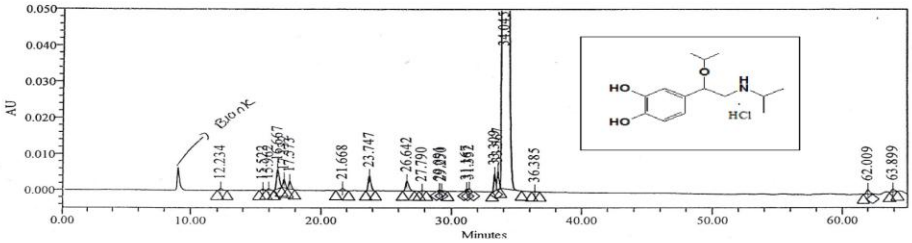
IR Spectrum of Compound 8



Mass Spectrum of Compound 8
S7

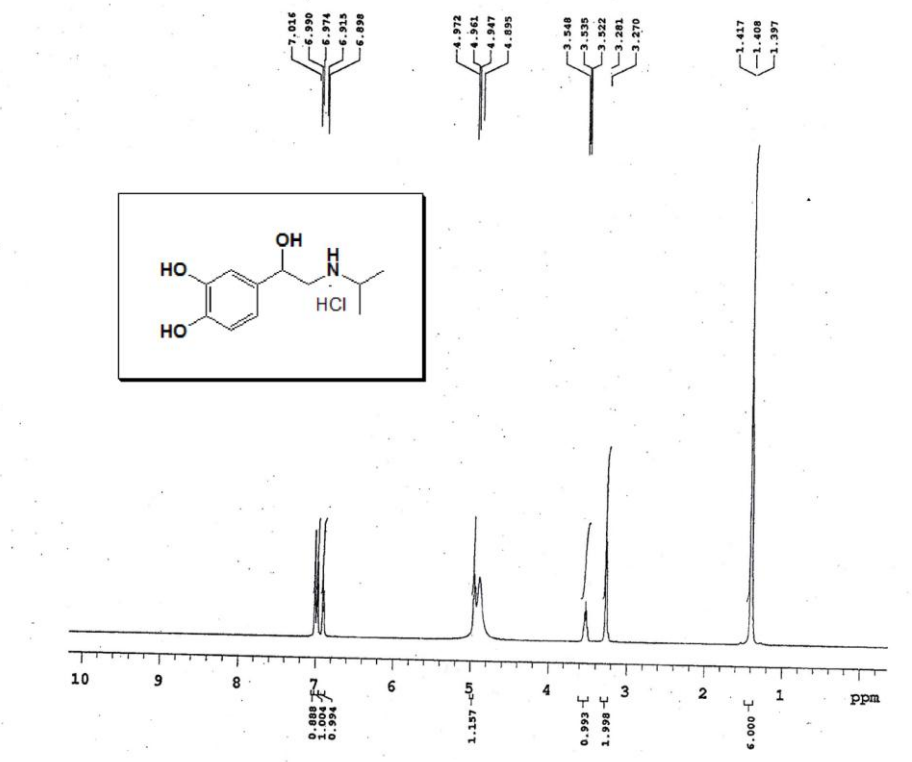


Elemental analysis Spectrum of Compound 8
S8

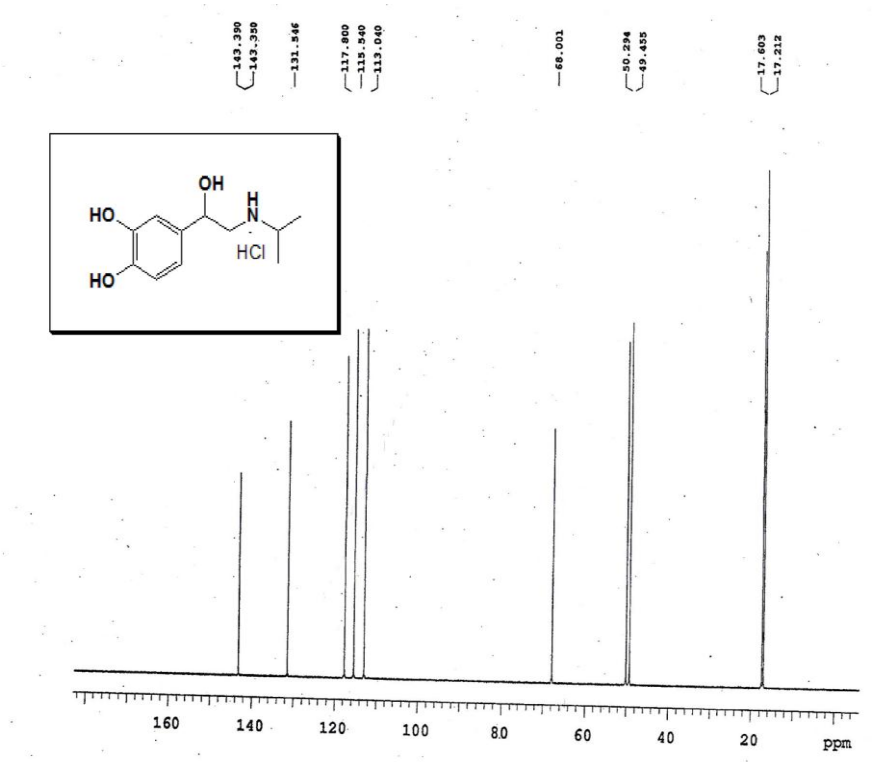


	Retention Time (min)	Area (µV*sec)	% Area	RT Ratio	Name
1	12.234	7247	0.09	0.36	Peak1
2	15.522	4952	0.06	0.46	Peak2
3	15.962	6911	0.09	0.47	Peak3
4	16.667	77630	0.99	0.49	Peak4
5	17.132	27946	0.36	0.50	Peak5
6	17.573	30029	0.38	0.52	Peak6
7	21.668	11976	0.15	0.64	Peak7
8	23.747	61477	0.78	0.70	Peak8
9	26.642	49108	0.62	0.78	Peak9
10	27.790	2441	0.03	0.82	Peak10
11	29.091	5489	0.07	0.85	Peak11
12	29.250	2738	0.03	0.86	Peak12
13	31.167	6543	0.08	0.92	Peak13
14	31.352	12634	0.16	0.92	Peak14
15	33.309	46051	0.59	0.98	Peak15
16	33.567	49387	0.63	0.99	Peak16
17	34.045	7416071	94.38		Isopropoxy isoproterenone
18	36.385	2043	0.03	1.07	Peak18
19	62.009	24710	0.31	1.82	Peak19
20	63.899	12610	0.16	1.88	Peak20

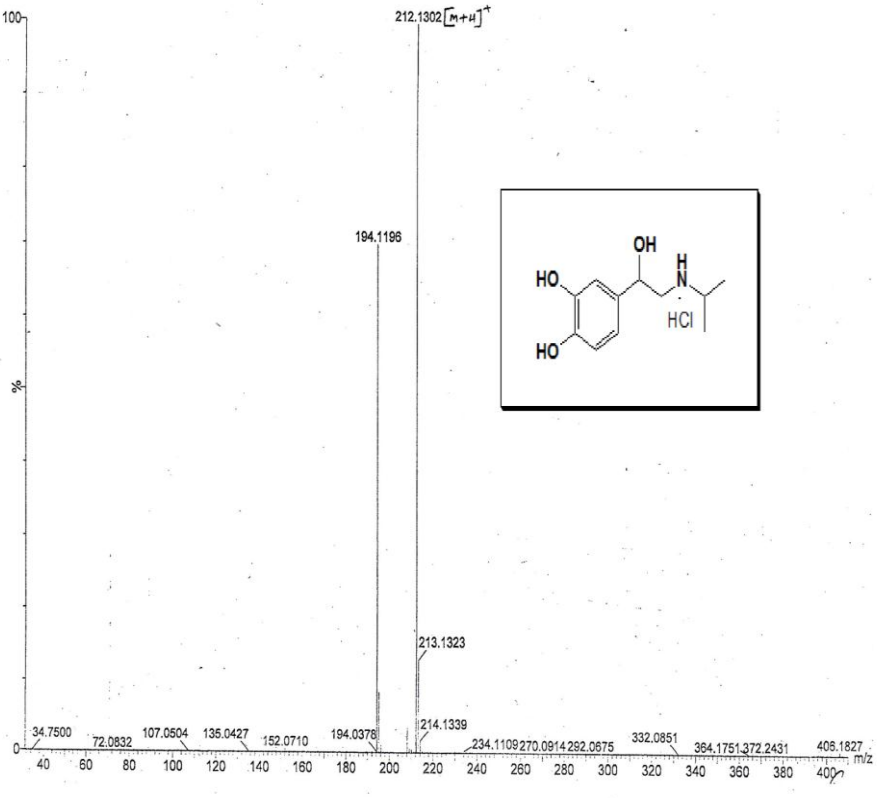
HPLC Spectrum of Compound 8
S9



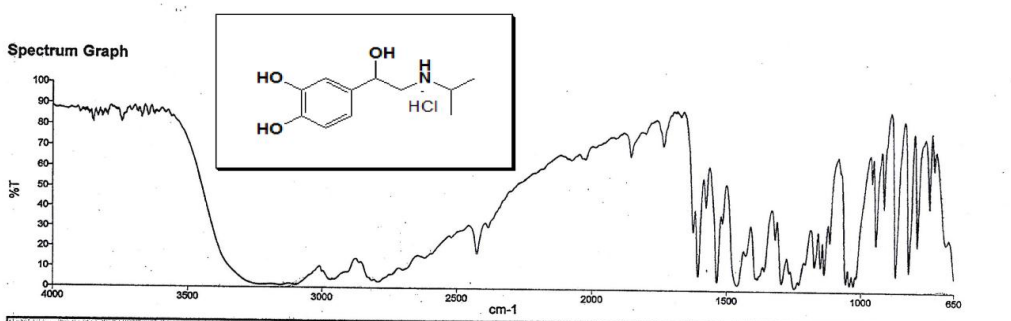
^{1}H NMR Spectrum of Compound 1
S 10



^{13}C NMR Spectrum of Compound 1
S 11



Mass Spectrum of Compound 1
S 12

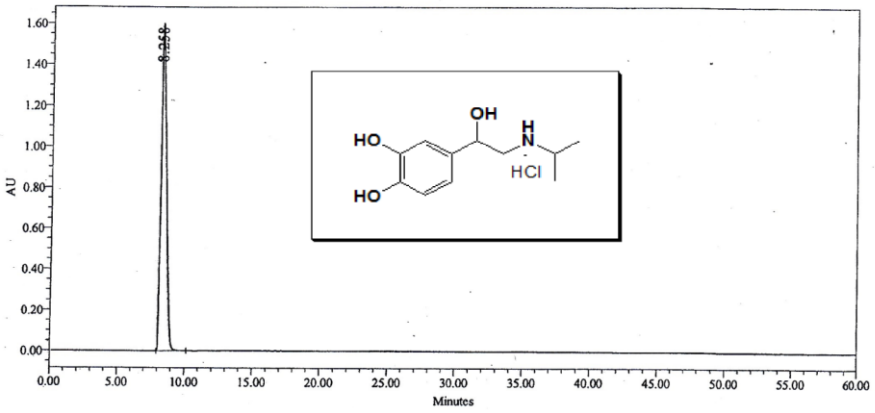


Name	Description
-	SAMPLE:ISOPROTERENOL HCl PROJECT:ISOPROTERENOL HCl 2.09mg OF SAMPLE IN 400.16mg KBr

Peak Table

Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)	Peak	X (cm-1)	Y (%T)
1	3901.68	85.693	2	3867.32	84.8999	3	3851.08	81.1434
4	3835.53	84.0815	5	3818.7	83.9275	6	3799.31	84.3859
7	3746.79	81.3804	8	3686.52	86.1726	9	3672.89	83.6221
10	3649.58	84.5577	11	3626.83	85.0618	12	3233.01	1.1977
13	3157.4	1	14	3101.01	1.0534	15	3001.71	7.7263

IR Spectrum of Compound 1
S 13



	Retention Time (min)	Area (µV*sec)	% Area	Int Type	Name
1	8.258	43791008	100.00	BB	Isoproterenol

HPLC Spectrum of Compound 1
S 14

CONCLUSION

In the present work, we have identified, characterized and synthesized two process related impurities of isoproterenol hydrochloride.

Supplemental Material

Full experimental detail, ^{1}H and ^{13}CNMR spectra, Mass, HPLC traces for this article can be accessed on the publisher's website.

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