Scientia Pharmaceutica www.scipharm.at

Research article Open Access

Isolation and Characterization of a Trace Level Unknown Impurity of Salmeterol by Chromatographic and Spectroscopic Methods

Bandaru VENKATASUBBAIAH * [1], Lakki Reddy PRAKASH [1], Rudraraju Vasu DEV [2], Vakamudi VARAPRASAD [3], Kalluru SUBRAMANYAM REDDY [4]

[1] Analytical Research and Development, Integrated Product Development, Dr. Reddy's Laboratories Ltd., Bachupally, Hyderabad-500 072, India.
[2] Analytical Research, Discovery Research, Dr. Reddy's Laboratories Ltd., Miyapur, Hyderabad-500 049, India.
[3] Research and Development, Integrated Product Development, Dr. Reddy's Laboratories Ltd., Bachupally, Hyderabad-500 072, India.
[4] Department of Chemistry, Sri Venkateswara University, Tirupati-517 502, India.

* Corresponding author. E-mail: venkatasb@drreddys.com (B. Venkatasubbaiah)

Sci Pharm. 2009; 77: 579–587 doi:10.3797/scipharm.0906-01

Published: July 4th 2009 Received: June 1st 2009
Accepted: July 2nd 2009

This article is available from: http://dx.doi.org/10.3797/scipharm.0906-01

Abstract

During the process development of Salmeterol, an unknown impurity was detected at 2.08 Relative Retention Time (RRT) at a level of 0.11% by a gradient Reverse Phase-High Performance Liquid Chromatography (RP-HPLC). The impurity was isolated from the salmeterol drug substance using preparative HPLC. The separation was achieved with an Inertsil C_8 column, using acetonitrile–trifluroacetic acid buffer pH 2.7 as mobile phase. The isolated impurity was characterized by NMR and MS techniques. The impurity has been characterized as 4-(2-{[6-(4-cyclohexylbutoxy)hexyl]amino}-1-hydroxyethyl)-2-(hydroxymethyl)phenol. The synthesis of the impurity and its formation was also discussed.

Keywords

Salmeterol Impurity • Preparative HPLC • Spectroscopy • Isolation • Identification and Characterization

Introduction

Salmeterol xinafoate (Serevent), 2-(hydroxymethyl)-4-(1-hydroxy-2-{[6-(4-phenylbutoxy)-hexyl]amino}ethyl)phenol – 1-hydroxynaphthalene-2-carboxylic acid (1:1), is a new long-acting β2-adrenoceptor agonist recently introduced for the treatment of asthma, which possesses both bronchodilator and antiinflammatory activity. The long duration of action of salmeterol, compared with other β2 adrenoceptor agonist drugs, such as salbutamol, probably results from its lipophilicity and its persistence at the site of action [1, 2]. Consequently, salmeterol may represent an important therapeutic advance in the treatment of asthma. Salmeterol xinafoate effectiveness has demonstrated at least of 12 hours in both adult [4] and pediatric [5] asthma patients. A number of large scale studies of adult [6–8] and pediatric [9, 10] asthma patients using Salmeterol have demonstrated improvement in overall lung function and control of symptoms with no harmful effects, as measured by methacholine challenge and the asthma exacerbation rate. The route of administration of salmeterol in clinical use is via inhalation. However, it is recognized that much of an inhaled dose of a drug is eventually swallowed [3] and can be absorbed into the systemic circulation from the gastrointestinal tract. Consequently, the oral route was one of the major routes used for the safety evaluation of salmeterol.

The stringent purity requirements from the International Conference on Harmonization (ICH) is all the impurities with more than 0.1% in the drug substance must be identified and characterized [11, 12]. Eight known impurities of salmeterol have been reported in USP monograph [13]. In the present process development batch, we have observed six reported impurities along with one unknown impurity (target impurity / impurity) and the HPLC chromatogram is shown in Figure.1. Herein we discuss the isolation and characterization of the target impurity in salmeterol. The structures of salmeterol and the target impurity are shown in Figure 2.

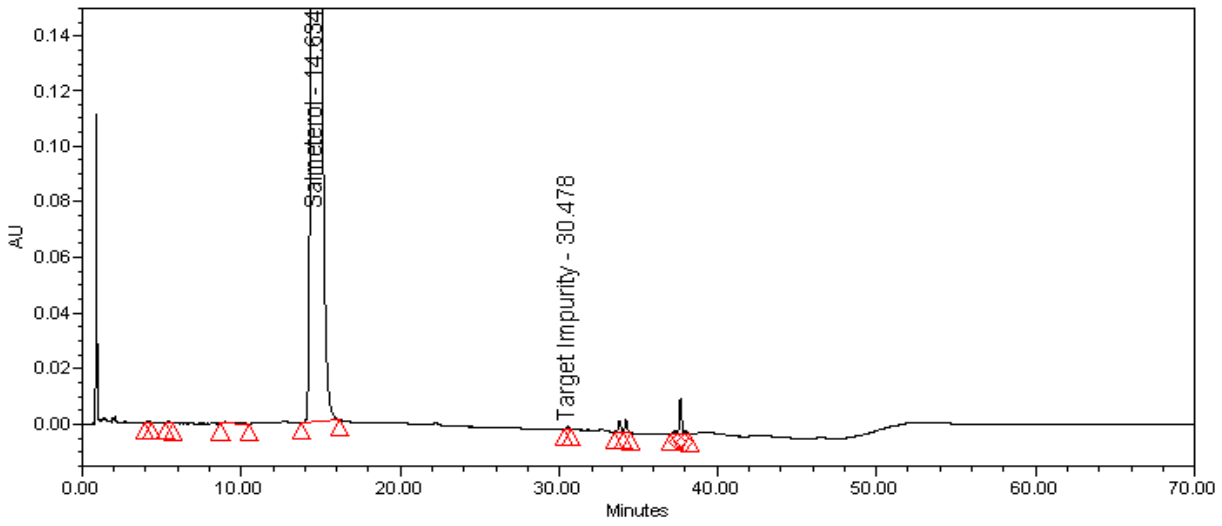


Fig. 1. HPLC chromatogram of Salmeterol drug substance.

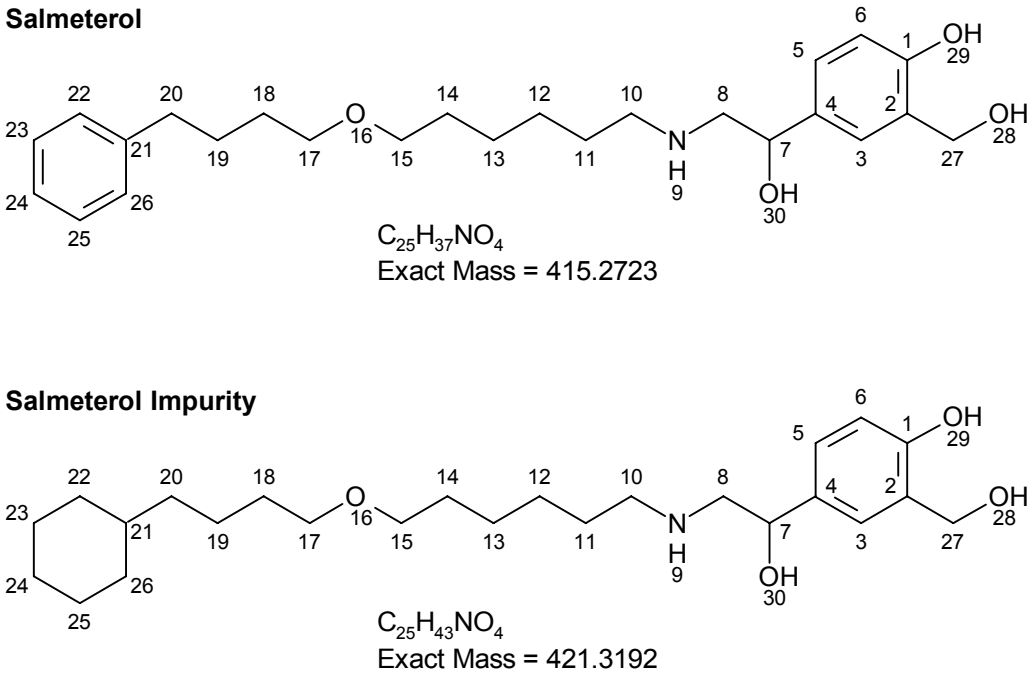


Fig. 2. Chemical structures of Salmeterol and the Target Impurity

Results and Discussion

Detection of impurity

A typical analytical LC chromatogram (Figure. 1) of a laboratory batch of Salmeterol bulk drug recorded using the LC method as described in the analytical method. The unknown impurity under study is marked as target impurity, which was eluted at retention time (RT) 30.48 min, while Salmeterol eluted at 14.63 min. The impurity was isolated from the mother liquor of Salmeterol by preparative HPLC.

Isolation of the impurity by preparative HPLC

The forced degradation studies couldn't' result in the enhancement of the target impurity. Various methods were tried to achieve the required resolution. Finally a reverse phase solvent system was developed with good resolution and reasonable runtimes. The impurity fractions were collected and checked the purity by analytical HPLC method. The fractions which have > 98% purity were pooled together and were concentrated under high vacuum on a Buchi Rotavapor Model R124. The impurity was isolated as a white powder solid having purity ~ 98%.

Structural elucidation of impurity

The NMR and high resolution Mass Spectroscopic (HR-MS) data of the impurity were compared with those of Salmeterol Base data ($C_{25}H_{37}NO_4$). The positive HR-MS data (Figure 3a) of impurity has exhibited protonated molecular ion $[M+H]^+$ at 422.3281, which corresponds to the molecular formula $C_{25}H_{44}NO_4$. The negative HR-MS data (Figure 3b) of impurity has exhibited deprotonated molecular ion $[M-H]^-$ at 420.3133, which corresponds to the molecular formula $C_{25}H_{42}NO_4$.The molecular formula of the impurity was deduced as

$C_{25}H_{43}NO_4$. The molecular formula shows that the impurity has six hydrogen atoms more than the parent. This can be attributed to the reduction of one aromatic ring.

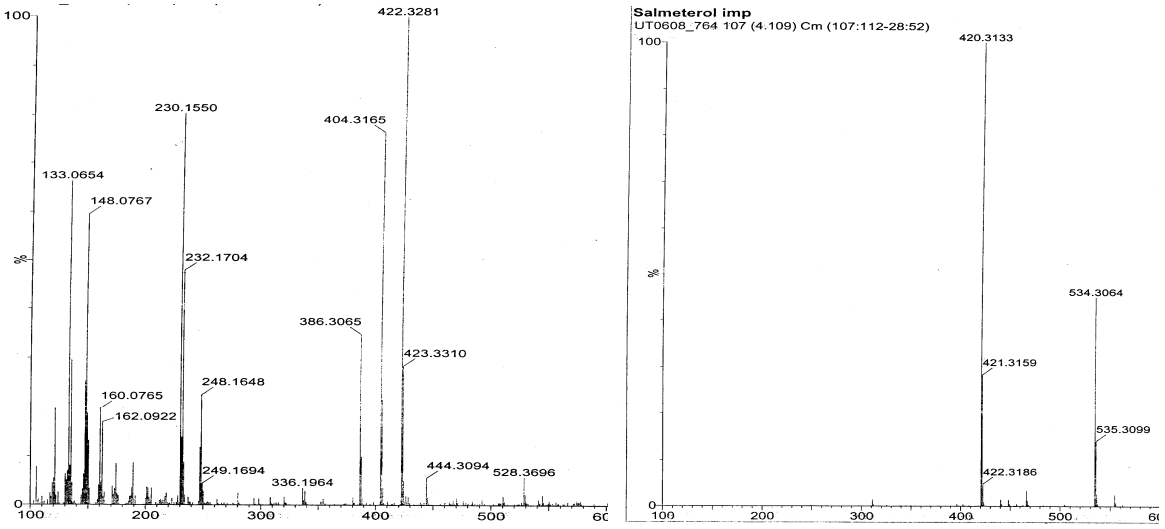


Fig. 3. +ve HR-MS of impurity (left); –ve HR-MS of impurity (right)

The proton NMR spectra of Salmeterol base (Figure 4) and impurity (Figure 5) are compared. The impurity ^{1}H NMR spectrum showed only three aromatic signals. The chemical shifts and splitting pattern showed that these protons correspond to 1, 2, and 4 substituted phenyl moieties. The aliphatic region between 0.8 ppm to 1.8 ppm was too crowded for the individual assignments. The crucial NMR assignments of the impurity are shown in Table-I along with salemterol base. The carbon chemical shifts are extracted from HSQC experiment.

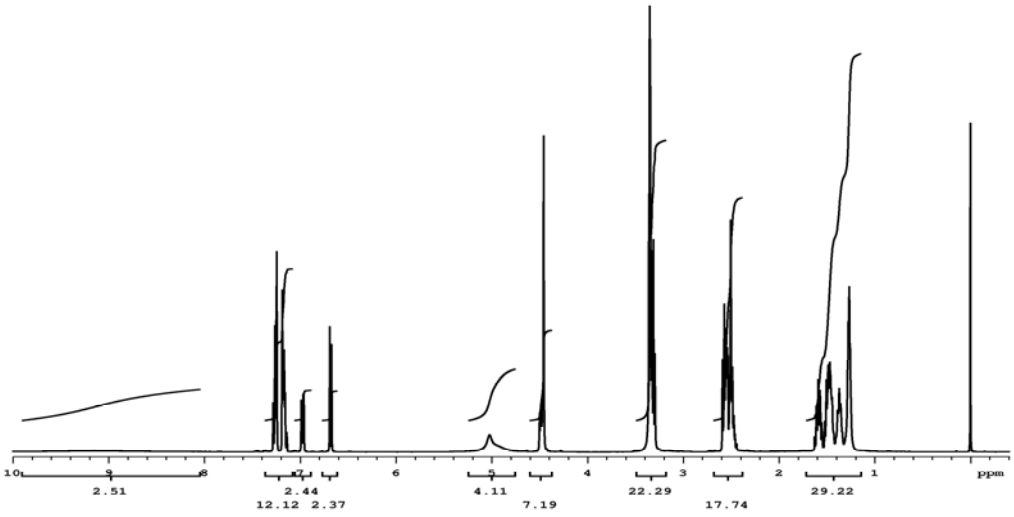


Fig. 4. ^{1}H NMR spectrum of Salmeterol base in $CDCl_3$

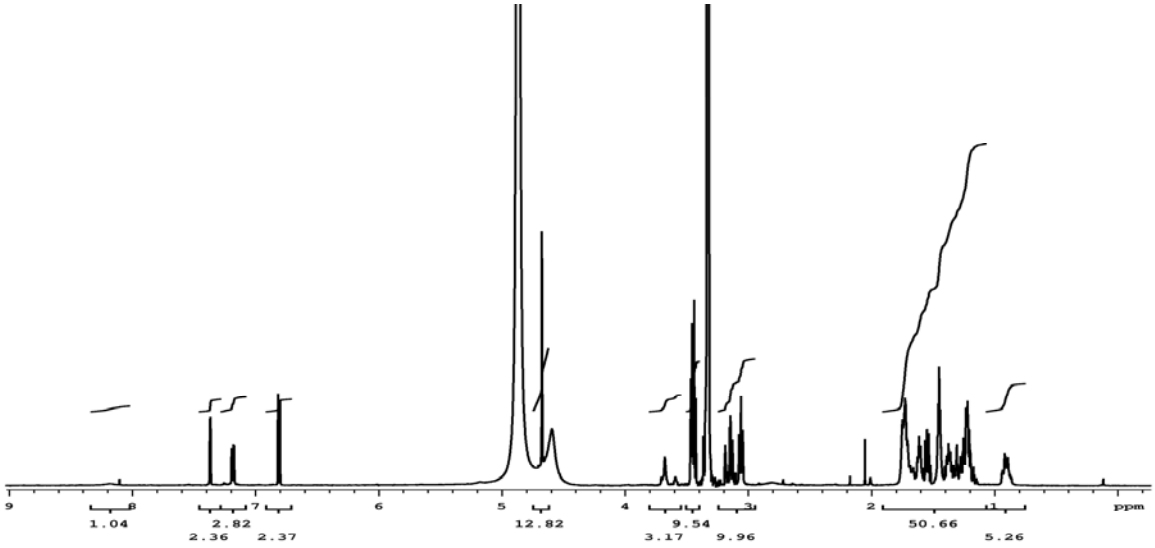
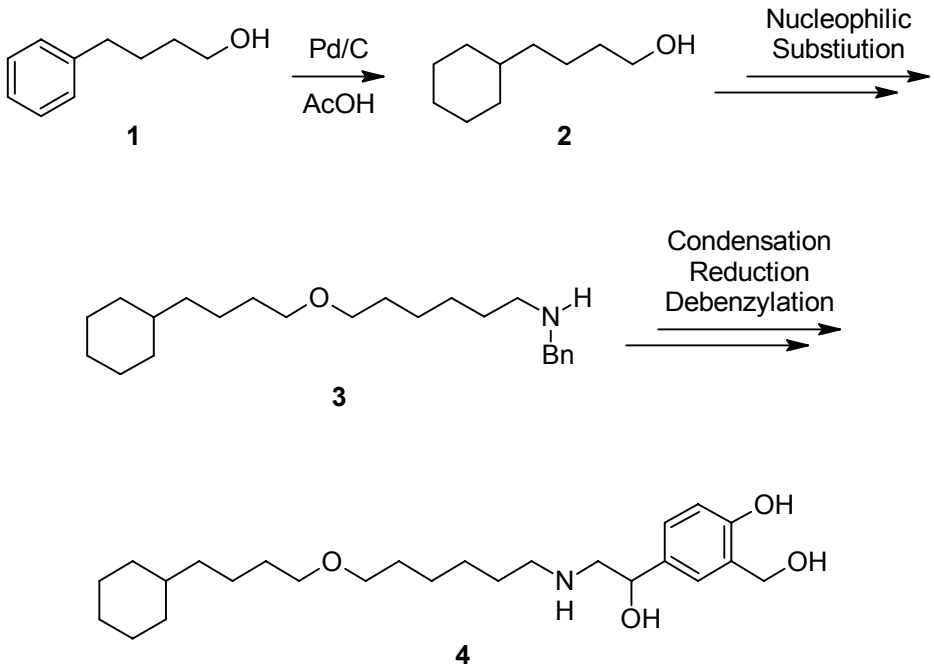


Fig. 5. ^{1}H NMR spectrum of Salmeterol impurity in CD_3OD

For further confirmation of the structure of the impurity, the MS/MS data of salmeterol base and the impurity were compared. The common fragments at m/z 232 and 248 supported the impurity structure arrived from MS and NMR data.

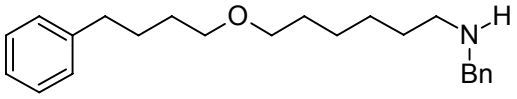
Based on the spectral data the impurity was identified as 4-(2-{[6-(4-cyclohexylbutoxy)-hexyl]amino}-1-hydroxyethyl)-2-(hydroxymethyl)phenol

Synthesis of salmeterol impurity



Sch. 1. Synthetic scheme of salmeterol impurity

This impurity forms in final API due to presence of *N*-benzyl-6-(4-cyclohexylbutoxy)hexan-1-amine (**3**) in key intermediate *N*-benzyl-6-(4-phenylbutoxy)hexan-1-amine. During the reaction sequence for preparation of sameterol; *N*-benzyl-6-(4-cyclohexylbutoxy)hexan-1-amine (**3**) undergoes analogous reactions as that of *N*-benzyl-6-(4-phenylbutoxy)hexan-1-amine (**5**), a key intermediate of our process, and finally leads to salmeterol impurity.



5

Fig. 6. *N*-benzyl-6-(4-phenylbutoxy)hexan-1-amine

Experimental

Samples and chemicals

The salmeterol drug substance was received from the R&D of Dr. Reddy's Laboratories Ltd., Hyderabad, India. The HPLC grade acetonitrile and methanol solvents were obtained from the Merck Co., Mumbai, India. Ammonium acetate was procured from the Merck India Ltd. Trifluoro acetic acid was procured from the ACROS organics, New Jersey, USA.

Analytical HPLC method

An Agilent HPLC system equipped with 1100 series low pressure quaternary gradient pump along with pulse dampener, photo diode array detector with auto liquid sampler handling system has been used for the analysis of samples. A symmetry shield RP18, 250 * 4.6 mm, 5-µm, column (Waters Ireland) was employed for the separation of impurities from Salmeterol. The column eluent was monitored at detection wavelength 278 nm. The gradient conditions employed for the separation with a timed gradient program of T (min)/%B (v/v): 01/0, 18/0, 36/70, 45/70, 50/0, 70/0. The initial mobile phase A was a mixture of 24 volumes of a 7.71 g/l solution of ammonium acetate with 24 volumes of a 28.84 g/l solution of sodium dodecyl sulphate and adjusted to pH 2.7 with glacial acetic acid and mixed with 52 volumes of acetonitrile. Mobile phase B was acetonitrile and milli Q water in a ratio of 95:5. Chromatography was performed at room temperature using a flow rate of 1.0 mL min-1.

High performance liquid chromatography (preparative)

WatersTM preparative HPLC was equipped with W 600 quaternary solvent delivery module Delta prep 2487 dual wavelength UV detector with auto liquid sampler handling system fitted with 5000µL loop. The gradient conditions employed for the separations with a timed gradient program of T (min)/%B (v/v): 01/0, 20/40, 30/40, 35/70, 38/0, 42/0. The initial mobile phase A was milli -Q water pH adjusted to 2.7 with trifluoro acetic acid. Acetonitrile was used as mobile phase B. Chromatography was performed at room temperature with 15 mL min-1 flow rate. UV detection was carried out at 278nm. The fractions having purity of more than 98% were collected and the acetonitrile was evaporated using rotavapor (Heidolph Laborota 4002 control) under high vacuum. The aqueous solution was subjected to lyophilization to obtain the solid impurity.

Tab. 1. NMR assignments of Salmeterol and Impurity

Atom[a]	^{1}H	Salmeterol Base			Impurity		
		δ (ppm)	J(Hz)[b]	^{13}C	δ (ppm)	J(Hz)[2]	^{13}C
1	–	–	–	153.00	–	–	155.23
2	–	–	–	125.02	–	–	127.98
3	1H	7.1–7.3	–	124.83	7.37	d(2.0)	125.74
4	–	–	–	134.61	–	–	131.61
5	1H	6.98	dd (8.5,2.0)	127.9	7.18	dd (2.0,8.5)	125.82
6	1H	6.68	d (8.5)	113.94	6.81	d (8.5)	114.84
7	1H	4.48	t (7.6)	71.35	4.80	–	68.90
8	2H	2.4–2.6	–	58.32	3.15	–	54.00
9	NH	4.8–5.2	br	–	–	–	–
10	2H	2.4–2.6	–	49.06	3.08	t (8.0)	47.65
11	2H	1.2–1.65	m	27.75	1.45–1.74	–	–
12*	2H	1.2–1.65	m	26.71	1.25–1.65	–	–
13*	2H	1.2–1.65	m	25.74	1.25	–	–
14#	2H	1.2–1.65	m	28.83	1.25–	–	–
15$	2H	3.31	m	69.71	3.45	–	70.64
17$	2H	3.31	m	69.93	3.46	–	70.64
18#	2H	1.2–1.65	m	29.26	–	–	–
19	2H	1.2–1.65	m	29.69	–	–	–
20	2H	2.4–2.6	t (8.0)	34.91	–	–	–
21	–	–	–	142.20	–	–	–
22,26	2H	7.26	m	128.28	–	–	–
23,25	2H	7.14–7.19	t (7.5)	128.24	–	–	–
24	1H	7.14–7.19	m	125.65	–	–	–
27	2H	4.46	s	58.32	–	–	–
28	OH	4.8–5.2	br	–	–	–	–
29	OH	9.0	br s	–	–	–	–
30	OH	4.8–5.2	br s	–	–	–	–

[a] Refer the Figure 2 for numbering. [b] This column gives the multiplicity and coupling constant: s-singlet, d-doublet, t-triplet, dd-doublet of doublet, m-multiplet and br-broad. *,$,# these assignments may interchange

UPLC-TOF

The UPLC-TOF-MS system consisted of an ACQUITY^{TM} Ultra Performance Liquid Chromatography system and a Micro mass LCT Premier XE Mass Spectrometer (High sensitivity orthogonal time-of-flight instrument, Waters, Modiford, USA) equipped with a lock mass sprayer, operating in either the positive or negative ion mode. All analyses were acquired using the lock spray to ensure accuracy and reproducibility; leucine-enkephalin was used as the lock mass. Sample of concentration 0.02mg/mL in methanol was infused

in TOF-MS at a flow rate of 10µL/min. High resolution (W mode, FWHM 10500) positive polarity scan responses were collected from m/z 100 to 1000 at a rate of 1.0 s/scan.

NMR spectroscopy

The NMR experiments were performed on Varian spectrometer operating at 400 MHz, Mercury plus, in DMSO-d_6 at 25°C. The proton and carbon chemical shifts were reported on δ scale in ppm, relative to TMS (δ=0.00 ppm) and DMSO (δ=39.50 ppm) as internal standard respectively. Standard pulse sequence provided by Varian was used for ^{1}H NMR.

Conclusion

This research paper describes the identification, isolation, structural elucidation and synthesis of an unknown impurity present in the Salmeterol. The impurity was isolated by preparative liquid chromatography. The isolated impurity was characterized by using spectroscopic techniques. The formation and synthesis of the impurity was also discussed in brief.

Acknowledgements

The authors thank the management of IPDO, Dr. Reddy's Laboratories for permitting this work to be published. Cooperation extended by all the colleagues of Analytical Research, at DR, is gratefully acknowledged.

The authors' Intellectual Property Management department (IPM) has given this manuscript the DRL-Publication No. IPDOIPM-00178.

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

References

[1] Brogden RN, Faulds D.
Salmeterol xinafoate. A review of its pharmacological properties and therapeutic potential in reversible obstructive airways disease.
Drugs. 1991; 42: 895–912.
doi:10.2165/00003495-199142050-00010

[2] Johnson M.
Salmeterol: a novel drug for the treatment of asthma.
Agents Actions Suppl. 1991; 34: 79–95.
PMid:1686530

[3] Newman SP, Woodman G, Clarke SW, Sackner MA.
Enhanced drug delivery from metered dose inhalers with Inspir Ease.
Am Rev Respir Dis. 1985; 131: A96.

[4] Bierman CW, Kemp JP, Sharpe MJ.
Salmeterol, a β2 agonist, induces bronchodilation for 12 hrs or greater: a placebo controlled single dose study.
J Allergy Clin Immunol. 1990; 85: 198.

[5] Verberne AA, Hop WC, Bos AB, Kerrebijn KF.
Effect of a single dose of inhaled salmeterol on baseline airway calibre and methacholine- induced airway obstruction in asthmatic children.
J Allergy Clin Immunol. 1993; 91: 127–134.
doi:10.1016/0091-6749(93)90305-Y

[6] Britton MG, Earnshaw JS, Palmer JB.
A twelve-month comparison of salmeterol with salbutamol in asthmatic patients.
Eur Respir J. 1992; 5: 1062–1067.
PMid:1426215

[7] Lundback B, Rawlinson DW, Palmer JB.
Twelve month comparison of salmeterol and salbutamol as dry powder formulations in asthmatic patients.
Thorax. 1993; 48: 148–153.
doi:10.1136/thx.48.2.148

[8] Wilding P, Thompson-Coon J, Clark M.
Efficacy and safety of long term treatment with salmeterol in adults with asthma.
Thorax 1995; 50 (Suppl 2): A39.

[9] Berdel D, Von Berg A, Scheinmann P.
Asthmatic children benefit from regular salmeterol xinafoate (50mcg tid) compared with salbutamol as required.
Eur Respir J. 1995; 8 (Suppl 19): 518S.

[10] Meijer GG, Postma DS, Mulder PG, van Aalderen WM.
Long-term circadian effects of salmeterol in asthmatic children treated with inhaled corticosteroids.
Am J Respir Crit Care Med 1995; 152: 1887–1892.
PMid:8520751

[11] ICH harmonized tripartite guideline.
Impurities in New Drug Substances Q3A(R2), current step 4 version dated 25 October 2006.

[12] ICH harmonized tripartite guideline.
Impurities in New Drug products Q3B(R2), current step 4 version dated 2 June 2006.

[13] USP Pharmacopeial Forum.
Volume No. 35(2), Page 307.