Journal of Pharmacy and Allied Health Sciences

ISSN 2224-2503

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Journal of Pharmacy and Allied Health Sciences 2 (1): 1-11, 2012
ISSN 2224-2503 / DOI: 10.3923/jpahs.2012.1.11
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Isolation, Characterization and Pharmacological Evaluation of 8-chloro-3 (1-methyl-piperidene-4-yl) 5H-benzo [5, 6] Cyclohepta [1, 2b] Pyridene-11(6H)-one from Loratadine

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ABSTRACT

The presence of impurities in the Active Pharmaceutical Ingredient (API) has always raised the curiosity of the synthetic or medicinal chemists. Thus the identification and understanding of these impurities has increased the interest of researchers and regulatory authorities all over the world. The present investigations were carried out on a process related impurity formed during the synthesis of loratadine. The impurity was isolated from an intermediate stage formed at the 9th stage of the synthesis (L-08) by column chromatography and analysed by HPLC, LC-MS, NMR and IR. The isolated compound was found to be a benzoic [5, 6] cyclohepta [1, 2b] pyridine and was identified to be a by product formed during the Grignard reaction involved in the loratadine synthesis. Further the impurity was subjected to anti-inflammatory and locomotor activity test to evaluate its pharmacological properties. The isolated compound showed potent anti-inflammatory activity, 58.6% at 5 mg kg^{-1} by Carageenan induced rat paw edema. Further the isolated compound was evaluated for locomotor activity and showed significant loss of locomotor activity at 50 mg kg^{-1}. The present findings showed that the impurity has significant pharmacological properties and its isolation from the API is a must in order to obtain a pure compound.

Key words: Anti-inflammatory, locomotor, benzo [5, 6] cyclohepta [1, 2b] pyridines, high performance liquid chromatography, liquid chromatography mass spectrometry

INTRODUCTION

Researchers have paid attention towards the growing need for controlling and formation of impurities in the drug substances and have been gaining critical importance from various regulatory authorities world over (Roy, 2002). The Webster's dictionary defines impurity as a "substance that is itself impure or makes something impure." Impurities are the unwanted compounds present in the pharmaceuticals, that develop during their synthesis and remain with the Active Pharmaceutical Ingredients (APIs). It may even develop during their formulation or upon aging of both API and formulated APIs to other dosage forms (Ahuja and Alsante, 2003). Their presence has an influence on the pharmacological action and the safety of the formulated product. There are different spectroscopic techniques to identify and characterize impurities i.e., NMR, Mass, IR and FTIR (Eugene *et al*., 2006). Finally, the compound can be identified by LCMS, HPLC-DAD-MS (Ermer, 1998; Gomez-Caravaca *et al*., 2007; Abdul *et al*., 2008). Generally these methods are quite complicated and require expensive instruments (Bhaskara *et al*., 2011).

Impurity identification is easier when carried out at stages earlier to the formation of the final API, i.e., at an intermediates stage. It helps in dealing with the impurity at the point of its formation which provides ample time to address various aspects of its formation and control. However the thresholds and guidelines of the International Council of Harmonization do not apply to the impurities at the developing stages (Ahuja and Alsante, 2003).

A wide variety of pollens, house dust mites and fungi are responsible for inducing allergic reactions in humans (Sharma *et al.*, 2009). These Allergic reactions are mainly mediated by histamine. It has been reviewed that histamine H_1 receptors are potential therapeutic targets of allergy and are most prominent in IgE mediated responses (Rahim, 2010). Antihistamines are the drugs which block the histamine messages from reaching their receptors. Loratadine is an antihistaminic drug belonging to the class of Second Generation Antihistamines. They are tricyclic antihistamines which are selective and inverse antagonists of histamine receptors. They are long lasting, non- sedative and peripherally acting. It has demonstrated effective control in allergic reactions over other antihistamines (Menardo *et al.*, 1997).

Loratadine was initially developed by Schering and Plough due to the arising need of a nonsedative antihistaminic drug. Its chemical structure closely relates to that of tricyclic antidepressants such as Imipramine and has distant structural similarity with atypical antipsychotic Quetiapine (Kay and Harris, 1999).

Much work has been done on Loratadine of which most of them have been carried out for pharmacokinetic studies and applied them to assess Loratadine and its metabolite Desloratadine in plasma by HPLC (Zhong and Blume, 1994), GC-MS (Martens, 1995) and GC with NP detector (Johnson *et al.*, 1994). It is highly essential to have a sensitive, reliable and at the same time a simple method for the determination of drugs in biological fluids for pharmacokinetic investigation (Jalalizadeh *et al.*, 2006).

Simultaneous determination of Loratadine and Desloratadine in plasma has been done by various researchers using methods like liquid chromatography and Tandem Mass Spectroscopy and HPLC with fluorescence detection (Yin *et al.*, 2003). The determinations of Loratadine impurities have been done by using various techniques like LCMS, preparative LCMS, preparative HPLC, UPLC etc. (Sutherland *et al.*, 2001). The analytical works were carried out for the purpose of validation of the methods like preparative LC, HPLC and Capillary Electrophoresis (Ruperez *et al.*, 2002). But the structures have not been characterized in this case. Impurities of loratadine have been isolated by using isocratic reverse phase HPLC from their mother liquor and then characterized using spectroscopic techniques (Reddy *et al.*, 2003). The usual hydroxy contaminant of Loratadine has also been synthesized and characterized (Veronica *et al.*, 2006). Some have been done to determine the amount of preservatives in Loratadine formulation by preparative TLC plates (Popovic *et al.*, 2007).

At the 9th stage of the synthesis, the conversion of 8-chloro 10,11-dihydro-4-aza-5H-dibenzo (a, d) cycloheptane-5-one (L-07) to 11-(N-Methyl-4-piperidinyl)-11-hydroxy-8-chloro-6,11-dihydro-5H-benzo (5, 6) cyclohepta (1, 2-b) pyridene (L-08) is mediated by Grignard Reaction. Methyl Piperidyl Magnesium Bromide acts as the Grignard reagent. It adds to L-07 under highly basic conditions created by Tetrahydrofuran and low temperature conditions ranging from -80 to -90°C (Suri *et al.*, 2004). At this stage quite a few impurities are formed out of which one is a critical impurity. This study is involved in isolation, characterization and evaluation of the critical impurity.

MATERIALS AND METHODS

Drugs and chemicals: various used drugs and chemicals include Acetonitrile, Isopropyl Alcohol, Ethyl acetate, Triethylamine, Methanol and Hexane obtained from S.D. Fine Chem. Ltd., Mumbai. Carageenan, Loratadine, Chlorpheniramine, Diclofenac Sodium were obtained from Spectrochem Pvt. Ltd., Mumbai. Crude samples of L-08 were obtained from Morepen laboratories Ltd., Parwanoo. All other chemicals were of analytical grade and were obtained from local sources.

Experimental animals: Wistar Alabino Rats of either sex weighing 110-150 g or Swiss Alabino mice weighing around 18-25 g were obtained from Guru Angad Dev Veterinary and Animal Sciences University. Animals were maintained at room temperature in standard cages under proper conditions and had free access to food and water. Twenty hours before the experiment the animals were starved however proper water supply was maintained. Animal experiments were carried out in accordance to the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals with registration No. 874/ac/05/CPCSEA.

Instruments used: IR was recorded on PERKIN ELMER BX FTIR spectrometer 882, ^{1}H NMR on a 400 MHZ FT NMR Spectrometer (Model BRUKER ADVANCE).

^{13}C NMR recording was done using 400 MHZ NMR Spectrometer (Model BRUKER ADVANCE) and LC-MS was on Finnegan Mat.

METHODS

Isolation of the impurity: To the Crude powdered sample of 11-(N-Methyl-4-piperidinyl)-11-hydroxy-8-chloro-6, 11-dihydro-5H-benzo (5, 6) cyclohepta (1, 2-b) pyridene (L-08) (70 g), 280 mL of Acetonitrile and 15 mL of water were charged. The mixture was then refluxed for an hour at temperatures ranging from 75 to 80°C. Then it was slowly cooled down for an hour upto 5°C for crystallization to take place. The crystals formed were separated by filtration and washed with a mixture of cold acetonitrile and water.

The filtrate and the residue were analysed for their impurity content. The filtrate was found to have excess of the impurity along with L-08 and most of the L-08 had been retained by the residue. Analysis was done by TLC (Isopropyl alcohol: Ethyl acetate: Triethylamine: Methanol /8:2:0.5:1). The filtrate was taken and the solvent was evaporated and dried. A small amount of the sample, about 20 g was taken and adsorbed on Silica Gel (100-200 mesh size) and loaded on to a column. The column was run using Hexane: Ethyl Acetate: Methanol (100: 2: 5) as the mobile phase and was run using gradient elution method. The elution was monitored by TLC. When pure spot of the impurity was observed in the TLC, the column fractions were collected and the excess solvent was evaporated at a temperature ranging from 50-60°C under a pressure of 200 mm Hg^{-1}.

PHARMACOLOGICAL EVALUATION

Anti-inflammatory activity: Carrageenan induced rat paw edema: The Carageenan induced rat paw edema assay was carried out according to the method of Winter *et al.* (1962).

The male alabino rats of either sex were randomly divided into four groups having four animals each:

- **Group I:** (Disease Control): Carageenan 1% in 0.9% Physiological Saline in the plantar surface of the rat
- **Group II:** (Standard): Diclofenac Sodium (5 mg kg^{-1}, p.o) in physiological saline

- **Groups III:** Test Compounds 2.5 mg kg^{-1} in physiological saline
- **Group IV:** Test Compounds 5 mg kg^{-1} in physiological saline

A solution of Carageenan (1%, 0.05 mL) in physiological saline injected into the subplantar tissues of right hind paw of the rat was used to induce edema. The phylogistic agent was injected one hour after the administration of the vehicle, test and the standard drug. The paw volume was measured at intervals of 1, 2, 3 and 4 h by mercury displacement method using a plethysmometer and the percentage inhibition of inflammation by the drug was calculated using the formulae given below:

$$\text{Inhibition of Edema (\%)} = (1-V_t/V_c)\times 100$$

Where:
V_t = Inflammatory increase in the rat paw volume of the rats of the treated group
V_c = Inflammatory increase in the rat paw volume of the rats of the control group

Spontaneous locomotor activity: Spontaneous Locomotor activity was carried out to evaluate the CNS depressant property of the impurity under study using an Actphotometer. Swiss Alabino mice of either sex were randomly divided into six groups of four animals each. Each animal was placed in the Actphotometer chamber individually for 25 min to note the basal readings:

- **Group I:** (Control) Treated with vehicle, physiological saline
- **Group II:** (Standard) Chlorpheniramine (10 mg kg^{-1}) (Vishwanatha *et al.*, 2011)
- **Group III:** Treated with Loratadine 10 mg kg^{-1}
- **Group IV:** Treated with Loratadine 50 mg kg^{-1} (Leza *et al.*, 1991)
- **Group V:** Administered test sample 10 mg kg^{-1}
- **Group VI:** Administered test sample 50 mg kg^{-1}

After thirty minutes of oral administration of vehicle, standard, Loratadine and test, the animals were placed individually in the Actphotometer for 25 min and their locomotor activity was noted. Percentage inhibition of locomotor activity for each group was calculated by using the formulae as follows (Verma *et al.*, 2010):

$$\text{Decrease in activity (\%)} = (1-W_a/W_b)\times 100$$

Where:
W_a = Average activity scores after administration
W_b = Average activity scores before administration

RESULTS AND DISCUSSION

Isolation: The residual impurity obtained was black in color and liquid in nature. The purity content of the sample was then analysed by HPLC which came out to be 70% pure. The sample was then characterized by spectroscopic techniques. The HPLC of the isolated impurity has been shown in Fig. 1.

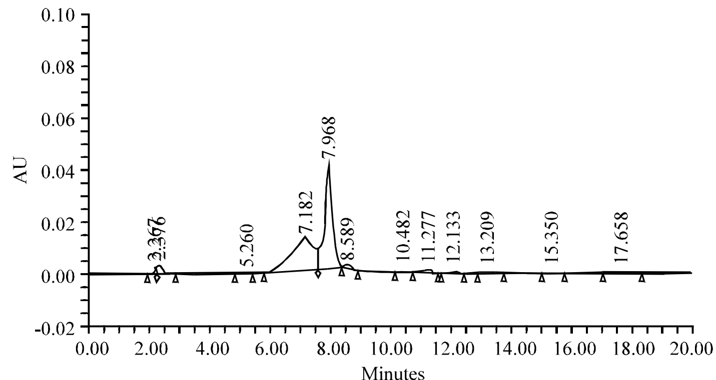


Fig. 1: HPLC showing the isolated impurity

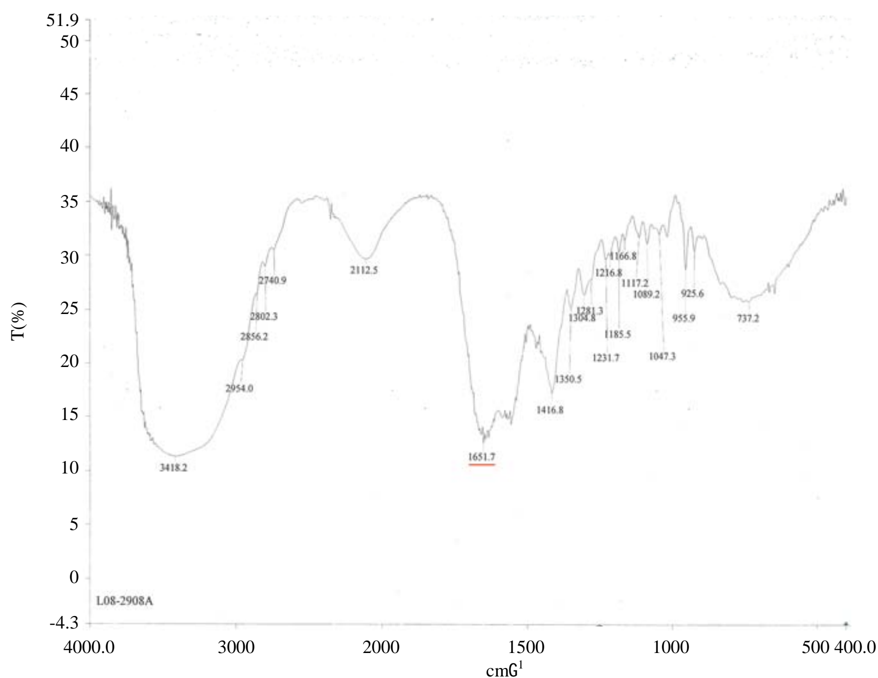


Fig. 2: IR Spectrum of the impurity

Characterization

IR (liquid): It shows characteristic peaks at 1651 cm^{-1} (C = C Stretch, C = O stretch), 1416 cm^{-1} ($-CH_3$ bend), 1350 cm^{-1} (C-N), 737.2 cm^{-1} (C-H aromatic oop.) (Fig. 2).

^{1}H NMR (400 MHZ $CDCl_3$): δ 8.5758-8.5707 (d, 1H, H_2) δ 8.04-8.02 (d, 1H, H_4) δ 7.48-7.47 (d, 1H, H_7) δ 7.337- 7.3110 (d, 1H, H_9) δ 7.27-7.25 (d, 1H, H_{10}) δ 2.66-2.96 (m, 4H, H_{5a}, H_{5b}, H_{6a}, H_{6b}), δ 2.42-2.43 (m, 3H, H_{22a}, H_{22b}, H_{22c}), δ 2.27-2.17 (m, 4H, H_{18a} H_{18b} H_{20a} H_{20b}) δ 2.03-1.87 (m, 5H, H_{16a}, H_{16b}, H_{17a}, H_{17b}, H_{21}).

^{13}C NMR (400 MHZ, $CDCl_3$): δ 32.81, 32.02, 34.32, 38.82, 45.53, 55.30, 127.43, 129.42, 132.9, 135.51, 135.85, 137.14, 138.81, 143.10, 148.10, 152.15, 192.7 (C = O).

Table 1: ^{1}H NMR data of (L-08)

Protons	δ (ppm)	^{1}H	J (Hz)
17,20,21	0.79-1.78	6H	m
22	2.17	3H	s
16	2.28-2.36	1H	m
18	2.73-2.81	2H	m
5	2.86-2.97	2H	m
6	3.41-3.54	2H	m
3,9,7,4	7.05-7.15	4H	m
23	7.43-7.45	1H	d
10	7.98-7.99	1H	d
2	8.27	1H	d

Table 2: ^{1}H NMR data of L-07

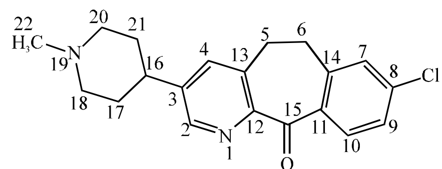
Positions	δ (pm)	^{1}H	J (Hz)
5H, 6H	3.4-3.16	4H	m
7H	7.17	1H	s
9H	7.25-7.27	1H	dd
3H	7.34-7.36	1H	dd
4H	7.61-7.63	1H	d
10H	7.96-7.98	1H	d
2H	8.63-8.64	1H	D

LC-MS: The major peaks were 341.1 (M^+), 342.1 (M^++2), 343.1 (M^++3).

Structure elucidation of impurity of loratadine: The LCMS for the impurity shows the molecular ion peak at 341.1 which is exactly one mass unit less than that of 11-(N-Methyl-4-piperidinyl)-11-hydroxy-8-chloro-6,11-dihydro-5H-benzo (5, 6) cyclohepta (1, 2-b) pyridene which is 342.9 (Fig. 4). Interestingly the proton NMR spectrum of the impurity shows five signals in the aromatic region which is one less than that present in the proton NMR spectrum of the L-08 (Table 1). Moreover the ^{13}C spectrum of the impurity shows similarity to the ^{13}C spectrum of L-07 (Fig. 5) in the aromatic region i.e., there is a high field absorption peak at 192 ppm in L-07 which is present in the spectrum of the impurity also indicating the presence of a ketone (Table 4). The other half of it shows similarity to that of L-08 (Table 3). The IR shows a peak at 1651 cm^{-1} which is similar to the absorption band present at 1664 cm^{-1} of L-07 which corresponds to a ketone (Table 6). All these observations indicate that there is the presence of dihydro-4-aza-5H-dibenzo (a, d) cycloheptane-5-one and N-Methyl-4-piperidine in the impurity but the point of attachment is different from that of L-08. The piperidene ring is not attached to the 11th position of the cyclic ring but somewhere in the aromatic region.

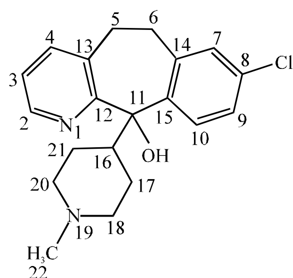
The structure of the aromatic ring of L-07 (Fig. 5) has three electronegative elements i.e. Chlorine Nitrogen and Oxygen thus giving rise to inductive effect and its data is presented in Table 2. Oxygen being the most electronegative of the three, its effect dominates. Thus due to the delocation of the charges and resonance effect, position 3 was found to be the most probable position for the attachment of the Piperidene ring. The structure elucidated has been shown in Fig. 3.

It was analysed that the impurity of Loratadine is formed due to the highly basic and low temperature conditions involved in the Grignard reaction during the addition of N-methyl-4-Chloro piperidene magnesium bromide i.e. the Grignard reagent to the 8-chloro 10,11-dihydro-4-aza-5H-



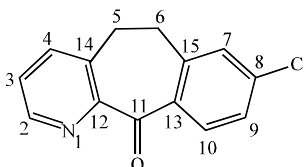
8-chloro-3-(1-methylpiperidin-4-y1)-5H-benzo[5,6]
cyclohepta[1,2-b]pyridin-11(6H)-one

Fig. 3: Structure of impurity



8-chloro-11-(1-methylpiperidin-4-y1)-6,11-dihydro-5H-benzo
[5,6]cyclohepta[1,2-b] pyridin-11-o1

Fig. 4: Structure of L-08



8-chloro-5H-[5,6]cuclohepta[1,2-b]pyridin-11()6H-one

Fig. 5: Structure of L-07

Table 3: ^{13}C Data of L-08

Carbons	δ (ppm)
17,21	26.16, 26.27
5,6	31.51, 32.60
16,22	44.82, 46.38
18,20	56.06, 56.40
11	77.93
3,4,7,8,9,10,13,14,15	122.95, 125.81, 129.80, 129.99, 132.42, 132.96, 138.06, 139.41, 141.56
2	143.94
12	158.38
$CDCl_3$	77.02,77.34,77.66

dibenzo(a,d)cycloheptane-5-one (L-07) (Fig. 5). L-07 is found to show Keto enol Tautomerism. IR spectroscopy data is shown in Table 5. In IR spectroscopy a normal ketone group shows stretching vibrations at 1751 cm^{-1} but when in conjugation with a double bond (C = C), the double bond absorb at lower frequency near 1675 cm^{-1} (Pavia *et al.*, 2007). In L-07, the ketone group absorbs at 1661 cm^{-1} and in the impurity it absorbs at 1651 cm^{-1}. It can be hypothesized that when the Grignard reagent comes in the vicinity of L-07 existing in the keto form the required product is

Table 4: ^{13}C Data of L-07

Carbons	δ (ppm)
5,6	32.17, 34.22
3,7	126.04,126.98
9,10	129.52, 132.54
13,14,15	135.45, 136.66, 137.47
4	138.64,
8	143.18
2	148.45
12	154.06
11	193.23
$CDCl_3$	77.16, 77.48, 77.80

Table 5: IR data of L-08

Frequency (cm^{-1})	Assignment
3229	O-H alcohols
2944, 2743	C-H alkane stretch
1248, 1276	C-O, C-N
874,834,723	C-H aromatic oop

Table 6: IR data of L-07

Frequency (cm^{-1})	Assignment
1664	C=O (Stretch)
1642,1583	C=C, C=N (Stretch)
837,792	C-H Aromatic

formed as in Grignard reaction. But in the case when L-07 exist in the enol form, there are chances that the Grignard reagent would attack the next most reactive point of the molecule, found to be the 3rd position which appears to be electron deficient due to the electronegative nitrogen atom.

ANIMAL ACTIVITIES

Anti-inflammatory activity

Carageenan induced rat paw edema: The test sample showed a pronounced effect on Carageenan induced edema, the maximum being 58.6% inhibition at 5 mg kg^{-1}. It showed a constant inhibition of inflammation all through the experiment. Edema inhibition was found to be dose dependant, more inhibition at higher dose of the test drug (5 mg kg^{-1}) than at lower doses (2.5 mg kg^{-1}) (Table 7).

The pharmacological evaluation of the molecule establishes that the impurity has potent anti-inflammatory activity. Carageenan induced rat hind paw edema is a common model used to evaluate acute inflammation and is believed to be biphasic. Reviewers have suggested that this model has good correlation between the efficacy in the model and human activity (Mohamed *et al.*, 2005; Garg and Paliwal, 2011). Anti-inflammatory activity carried out for pyridenedicarbonitrile and benzopyranopyridenes showed very high anti-inflammatory activity (Hosni and Abdulla, 2008).

Spontaneous locomotor activity: Locomotor activity exhibited significant decrease in case of standard drug Chlorpheniramine. Loratadine did not show any decrease in motility as it is a non sedative antihistaminic drug. The test sample at 50 mg kg^{-1} did show major CNS depression while dosage at 10 mg kg^{-1} showed less CNS depression (Table 8).

Table 7: Anti-inflammatory activity of the Impurity

Groups	Dosage	1 h	2 h	3 h	4 h	% inhibition after 4th h
Control	Normal saline	0.69±0.011	0.65±0.044	0.71±0.021	0.75±0.07	--
Diclofenac sodium	5 mg kg^{-1}	0.435±0.02^c	0.405±0.04^b	0.315±0.01^a	0.275±0.06^a	63.3
Test sample	2.5 mg kg^{-1}	0.55±0.02^a	0.50±0.04^b	0.49±0.06^b	0.43±0.05^c	42.6
Test sample	5 mg kg^{-1}	0.43±0.016^c	0.40±0.03^a	0.38±0.013^c	0.31±0.04^b	58.6

Values are Mean±SEM for 4 animals in each group. a = p<0.01, b = p<0.02, c = p<0.05 as compared to control, using one way ANOVA

Table 8: Spontaneous locomotor activity of the impurity

Groups	Dosage	Basal readings	After treatment	% inhibition
Control	Normal saline	501.00±55	531.75±45	--
Chlorpheniramine	10 mg kg^{-1}	571.25±13.5	103.11±10	80.80
Loratadine	10 mg kg^{-1}	557.25±10	526.86±18.45	--
Loratadine	50 mg kg^{-1}	550.00±29.5	516.46±11.15	--
Test sample	10 mg kg^{-1}	522.75±17.5	319.50±9.25	39.91
Test sample	50 mg kg^{-1}	601.50±18.5	259.56±20.32	51.18

Values are Mean±SEM for 4 animals in each group. p<0.01 using one way ANOVA

The impurity causes remarkable CNS depression at doses higher than 10 mg kg^{-1}. The locomotor activity using the Actophotometer measures the level of excitability of the central neurons and a decrease in activity may be due to sedation resulting from the depression of the CNS (Balamurugan *et al*., 2007; Pandy *et al*., 2009). Locomotor activity was studied on synthesized derivatives of benzocycloheptane pyridene derivatives. The derivatives showed considerable decrease in locomotor activity as compared to the standard drugs (Carceller *et al*., 1994). The Structure Activity Relationship of Loratadine states that the piperidene ring along with ethyl ester molecule attached to the cycloheptane ring is responsible for the prevention of the molecule from entering the blood brain barrier. In this case sedative properties attributes to the non attachment of the piperidene ring to the cycloheptane ring (Wilson *et al*., 2004).

CONCLUSION

The results and discussions briefed above indicate that the impurity is a critical one and should be removed as it can influence the pharmacological profile of the drug. It also bears the chances of changing into other products during the course of the synthesis.

It can be suggested that the formation of this impurity can be reduced by decreasing the amount of tetrahydrofuran which would decrease the basicity of the medium and by increasing the temperature conditions from -90^{0}C to 10^{0}C during the synthesis of L-08.

ACKNOWLEDGMENTS

Sincere and heartfelt thanks are extended to the Research team of Research and Development Lab of the Morepen Laboratories Limited for making all their facilities available to carry out this work successfully and for their financial support.

REFERENCES

Abdul, A.B.H., A.S. Al-Zubairi, N.D. Tailan, S.I.A. Wahab, Z.N.M. Zain, S. Ruslay and M.M. Syam, 2008. Anticancer activity of natural compound (Zerumbone) extracted from *Zingiber zerumbet* in human hela cervical cancer cells. Int. J. Pharmacol., 4: 160-168.

Ahuja, S. and K.M. Alsante, 2003. Hanbook of Isolation and Characterization of Impurities in Pharmaceuticals. 1st Edn., Academic Press, California, pp: 13-20.

Balamurugan, K.B., A. Dey and G. Sivakumar, 2007. Some neuropharmacological effects of the crued extract of *Conus parvatus* in mice. Pak. J. Biol. Sci., 10: 4136-4139.

Bhaskara, B.L., S.A. Kumar and U.R.A. Kumar, 2011. A facile and rapid HPLC method for the determination of atenolol in pharmaceutical formulations. Asian J. Applied Sci., 4: 306-313.

Carceller, E., M. Merlos, M. Giral, D. Balsa and C. Almansa *et al.*, 1994. [(3-Pyridylalkyl) piperidylidenel]benzocycloheptapyridine derivatives as dual antagonists of PAF and histamine. J. Med. Chem., 37: 2697-2703.

Ermer, J., 1998. The use of hyphenated LC-MS technique for characterization of impurity profiles during drug development. J. Pharm. Biomed. Anal., 18: 707-714.

Eugene, P.K., R.D. Gregg, A.B. Kelly, P. Charles, S. Tyrone, J.Y. Henry and Z. Zhongli, 2006. Quantitative HPLC tandem mass spectrometry (LC/MS/MS) analysis of low- level impurities in active pharmaceutical ingredient. Am. Pharm. Rev., 4: 82-87.

Garg, V.K. and S.K. Paliwal, 2011. Anti-inflammatory activity of aqueous extracts of *Cynodon dactylon*. Int. J. Pharmacol., 7: 370-375.

Gomez-Caravaca, A.M., L. Cerratani, A. Bendeni, A. Segura-Carretero, A. Fernandez-Gutierrez and G. Lercker, 2007. Effect of filtration systems on the phenolic content of virgin olive oil by HPLC-DAD-MSD. Am. J. Food Technol., 2: 671-678.

Hosni, H.M. and M.M. Abdulla, 2008. Anti-inflammatory and analgesic activities of some newly synthesized pyridinecarbonitrile and benzopyranopyridene derivatives. Acta Pharma., 58: 175-186.

Jalalizadeh, H., E. Souri, H.R.J. Ghanami, A. Almasirad and A. Foroumadi, 2006. A rapid and sensitive HPLC method for the determination of 2-hydroxyflutamide in human plasma. Int. J. Pharmacol., 2: 221-225.

Johnson, R., J. Christensen and C.C. Lin, 1994. Sensitive Gas-liquid chromatographic method for the determination of loratadine and its major active metabolite descarboethoxyloaratadine in human plasma using a nitrogen-phosphorous detector. J. Chrom. B: Biomed. Sci. Appl., 657: 125-131.

Kay, G.G. and A.G. Harris, 1999. Loratadine: A non-sedating antihistamine. Review of its effects on cognition, psychomotor performance, mood and sedation. Clin. Exp. Allergy., 29: 147-150.

Leza, J.C., I. Lizasoain and P. Lorenzo, 1991. Effect of antihistaminics on locomotor activity in mice. Comparison with opiate and amphetamine-induced hyperactivity. Gen. Pharmacol., 22: 293-296.

Martens, J., 1995. Determination of loratadine and pheniramine from human serum by gas chromatography-mass spectrometry. J. Chromatogr. B., 673: 183-188.

Menardo, J.L., F. Horak, M.R. Danzig and W. Czarlewski, 1997. A review of loratadine in the treatment of patients with allergic bronchial asthama. Clin. Ther., 19: 1278-1293.

Mohamed, M.S., I.M. Kamal, A.A. Mahmoud, A.H.S. Gaber and M.A. Mostafa, 2005. Synthesis and anti-inflammatory evaluation of some new quinazoline derivatives. Int. J. Pharmacol., 1: 261-266.

Pandy, V., N. Jose and H. Subhash, 2009. CNS activity of methanol and acetone extracts of *Acorus calamus* leaves in mice. J. Pharmacol. Toxicol. 4: 79-86.

Pavia, D.L., G.M. Lampman, G.S. Kriz and J.R. Vyvyan, 2007. Infrared Spectroscopy. In: Spectroscopy, Pavia, D.L., G.M. Lampman, G.S. Kriz and J.R. Vyvyan (Eds.). 1st Edn. Cengage Learning, New Delhi, India, ISBN-13: 9788131505762, pp: 30-38.

Popovic, G., M. Cakar and D. Agaba, 2007. Simultaneous determination of loratadine and preservatives in syrups by thin layer chromatography. Acta Chromatogr., 19: 161-169.

Rahim, F., 2010. An in silico development of selective inhibitor for histamine receptors. Biotechnology, 9: 157-163.

Reddy, K.V., J.M. Babu, Y.R. Kumar, S.V. Reddy and M.K. Kumar *et al.*, 2003. Impurity profile study of loratadine. J. Pharm. Biomed. Anal., 32: 29-39.

Roy, J., 2002. Pharmaceutical impurities-A mini review. AAPS Pharmscitech., 3: 1-8.

Ruperez, F.J., H. Fernandez and C. Barbas, 2002. LC determination of loratadine and related impurities. J. Pharm. Biomed. Anal., 29: 35-41.

Sharma, D., B.K. Dutta and A.B. Singh, 2009. Pollen, fungus and house dust mites survey at the residence of 90 allergic patients in greater silchar area of Assam, India. Res. J. Allergy, 1: 1-11.

Suri, S., N. Singh and S.S. Naim, 2004. Process for the preparation of loratadine. Patent Cooperation Treaty, Publication No. WO2004/080997A1.

Sutherland, F.C., A.D. de Jager, D. Badenhorst, T. Scanes, H.K. Hundt, K.J. Swart and A.F. Hundt, 2001. Sensitive liquid chromatography-tandem mass spectrometry method for the determination of loratadine and its major active metabolite descarboethoxyloratadine in human plasma. J. Chrom. A., 914: 37-43.

Verma, A., G.K. Jana, S. Sen, R. Chakraborty, S. Sachan and A. Mishra, 2010. Pharmacological evaluation of *Saraca indica* Leaves for central nervous system depressant activity in mice. J. Pharm. Sci. Res., 2: 338-343.

Veronica, C., M.P. Matia-Martín, J.L. Novella and J. Alvarez-Builla, 2006. Synthesis of 2-and -4 hydroxymethyl loratadine, usual impurities in Loratadine syrup formulations. Arkivok, 9: 200-206.

Vishwanatha, G.L., B.J. Priyadarshini, N. Krishnadas, S. Janardhanan, S. Rangappa and S. Hanumanthappa, 2011. Synthesis and Antihistaminic activity of 3H-benzo [4,5] thieno [2,3-d][1,2,3] triazin-4-ones. Saudi. Pharm. J.

Wilson, C.O., J.H. Block, J.M. Beale and O. Gisvold, 2004. Histamine and Antihistaminic Agents. In: Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, Beale, J.M. and J.H. Block (Eds.). Lippincott Williams and Wilkins, Philadelphia, pp: 713-714.

Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Carageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544-547.

Yin, O.Q.P., X. Shi and M.S.S. Chow, 2003. Reliable and specific high-performance liquid chromatographic methods for simulatanous determination of loratadine and its metabolite in human plasma. J. Chrom., 796: 165-172.

Zhong, D. and H. Blume, 1994. HPLC determination of loratadine and its active metabolite descarboethoxyloratadine in human plasma. Pharmazie, 49: 736-739.