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Short communication

Application of LC–MS/MS for the identification of a polar impurity in mosapride, a gastroprokinetic drug

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Abstract

In the impurity profile of mosapride a polar impurity (0.1%) was detected in HPLC with respect to mosapride. Based on the mass spectral data obtained by LC–MS/MS analysis this impurity structure was characterised as 4-amino-5-chloro-2-ethoxy-N-[[(4-benzyl)-2-morphinyl] methyl] benzamide. It is interesting to note that this impurity is potent analogue of mosapride, which will have much higher gastroprokinetic activity than metoclopramide. This impurity was synthesised from an unambiguous route and confirmed the structure by collecting various spectral data and the formation is discussed. To our knowledge this compound was not reported as process impurity elsewhere.

Keywords: Mosapride; Impurity; Spectroscopy; Identification; Liquid chromatography-Mass spectrometry; Characterization; Synthesis

1. Introduction

Gastrointestinal (GI) motility dysfunctions associated with non-ulcer dyspepsia (NUD), gastro oesophageal reflux disease and gastroparesis are known to be effectively treated with gastroprokinetic agents, such as metoclopramide, cinitapride and cisapride, which active serotonin 5-HT4 receptors to enhance acetyl choline release from the mysenteric plexus of the gut. However, these agents especially metoclopramide, which block dopamine D2 receptors cause several adverse effects such as central nervous system depression and extra pyramidal syndrome in man [1–3]. Mosapride citrate was found to be a potent gastroprokinetic agent with selectivity for 5-HT4 receptor [4,6]. In addition, the pharmacological profile of mosapride is different from that of cisapride and metoclopramide. Mosapride is free of dopamine D2 receptor antagonist property whereas the latter two agents possess it. Unlike cisapride it does not stimulate colonic motor activity [5]. It is a stringent regulatory requirement

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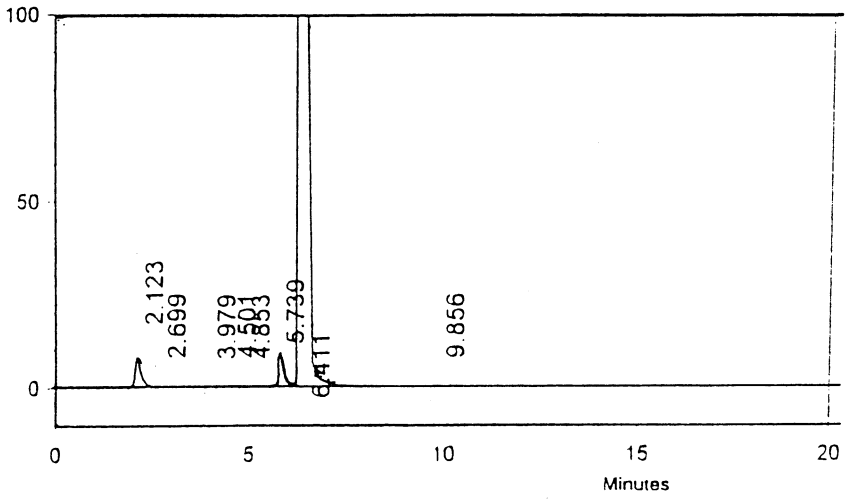


Fig. 1. LC chromatogram of mosapride citrate.

that all the impurities $\geq 0.1\%$ must be identified and characterised. This paper aims at the identification and characterisation of potential and prominent impurity by LC–MS/MS, that is present at a level of $< 0.1\%$ in the bulk drug of mosapride. Several batches of the mosapride citrate produced by Dr. Reddy's Holdings Ltd. have only one prominent impurity, which is the objective of present study. In all the batches this impurity range from 0.1 to 0.05%. Mosapride optical resolution was observed in the literature [7].

2. Experimental

2.1. Samples and chemicals

The pharma grade sample of mosapride and the synthesised impurity were obtained from Dr. Reddy's Holdings Ltd (which is sister concern of Dr. Reddy's Laboratories Ltd), Hyderabad, India. HPLC grade acetonitrile was obtained from Merck Co. (Mumbai, India). Potassium dihydrogen ortho phosphate, ammonium acetate salt, glacial acetic acid and orthophosphoric acid were procured from Qualigens (Mumbai, India). Tri ethylamine was procured from Fluka, Switzerland.

2.2. HPLC

A Shimadzu HPLC equipped with LC-10AD pump and SPD-M10AVP diode array detector have been used. A symmetry shield (250 × 4.6 mm, 5 μ, Waters, USA) column was used for the separations. The column eluent was monitored at 274 nm. The mobile phase was a mixture of 0.025 M buffer KH_2PO_4, triethylamine and acetonitrile (50:0.5:50). Buffer was prepared by dissolving 3.40 g of potassium dihydrogen phosphate in 1000 ml of water, mixing with 0.5 ml of triethylamine and pH adjusted to 4.0 with phosphoric acid. The mobile phase was filtered through a nylon membrane (pore size 0.45 μm). Chromatography was performed at room temperature using at a flow rate of 1.0 ml min^{-1}.

2.3. Mass spectrometry

LC–MS/MS analysis has been performed on API 3000 PE Sciex mass spectrometer. The analysis done in positive ionisation with Turbo ion spray interface with the following conditions. Ion source voltage IS = 5000 V, declustering potential, DP = 80 V, entrance potential = 300 V with nebuliser gas as air at 8 psi and curtain gas as nitrogen at 8 psi. A Hichrom (250 × 4.6 mm, 5 μ, Hichrom Ltd, UK) column was used for the

separation. The mobile phase was a mixture of 0.01 M buffer CH_3COONH_4, and acetonitrile (50:50). Buffer was prepared by dissolving 0.77 g of ammonium acetate in water and pH adjusted to

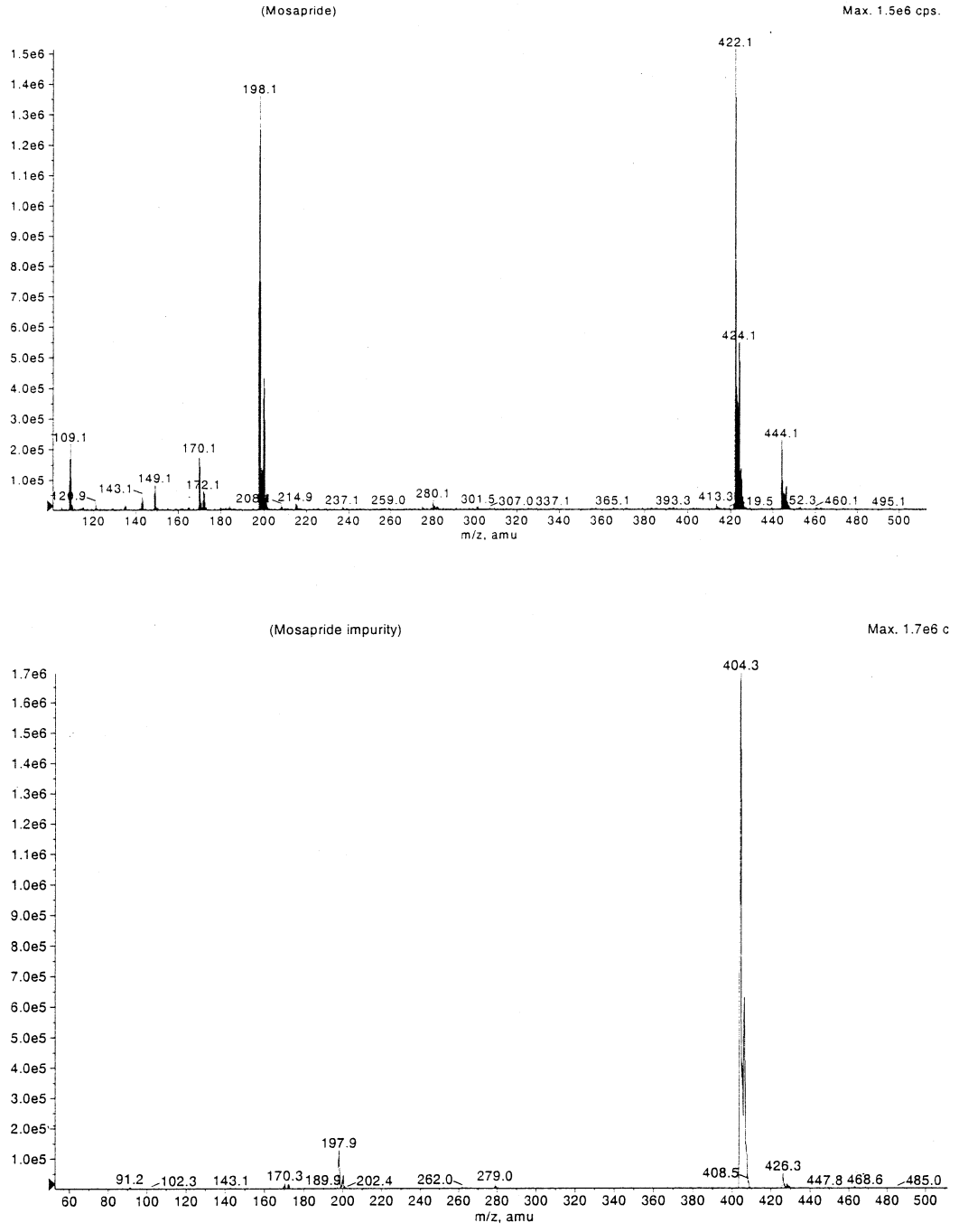


Fig. 2. Mass spectra of mosapride and impurity.

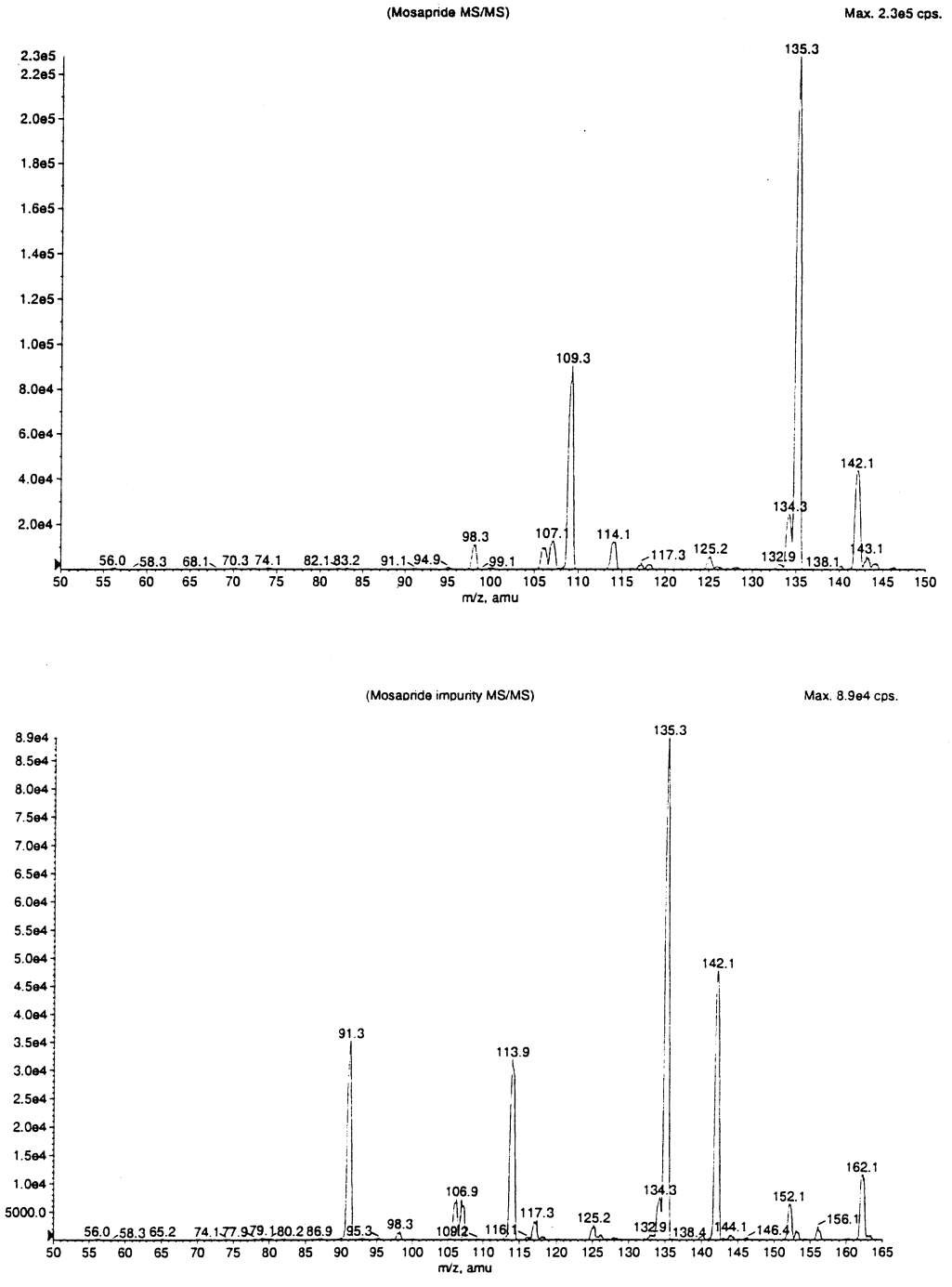
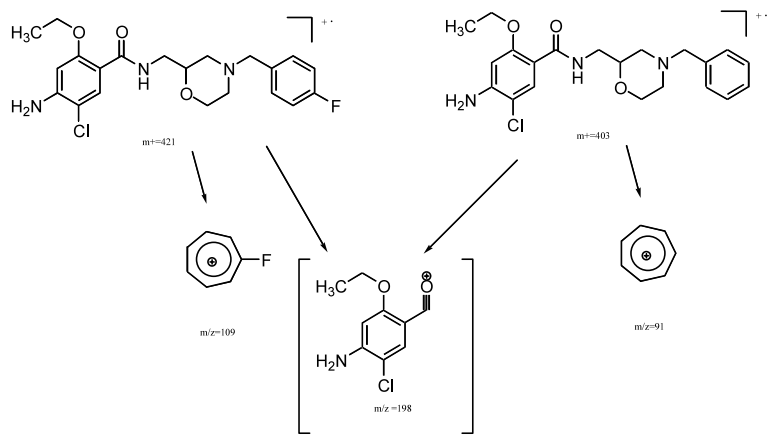


Fig. 3. MS/MS spectra of mosapride and impurity.



Mosapride	Mosapride impurity
$M^+ = 421$	$M^+ = 403$
m/z = 198, 170, 142,135 and 109	m/z = 198,170, 142, and 91

Fig. 4. Mass fragmentation path way for mosapride and impurity.

4.0 with acetic acid. The analysis was performed at a flow rate of 1.0 ml min^{-1}.

2.4. NMR studies

The ^1H and ^{13}C NMR studies were done at 400 and 50 MHz, respectively, in $CDCl_3$ on a Varian Gemini 400 and 200 MHz FT NMR spectrometers, respectively. ^1H and ^{13}C chemical shifts are reported on the δ scale in ppm, relative to tetra methyl silane (TMS) (δ 0.00) and $CDCl_3$ (δ 77.0), as internal standard, respectively. Distortionless enhancement polarisation transfer (DEPT) spectral editing revealed the presence of methyl and methine groups as positive peaks while the methylenes as negative peaks. The exchangeable protons were identified by D_2O exchange.

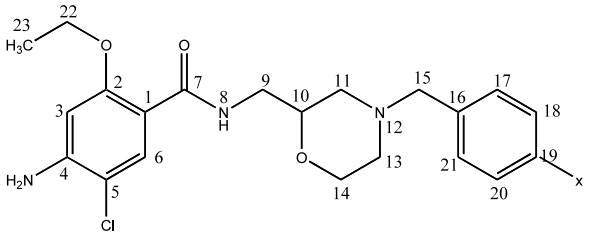


Fig. 5. Structural formula of mosapride and impurity.

2.5. FT IR spectroscopy

The IR spectra for mosapride, and its impurity were recorded in the solid state as KBr dispersion using Perkin Elmer 1650 FT IR spectrophotometer.

3. Results and discussion

3.1. Detection and identification of impurity

The HPLC analysis of mosapride citrate showed a polar impurity at 0.89 RRT (> 0.1%), where as the citric acid eluted at 0.33 RRT (Fig. 1). A mass compatible solvent system as discussed in Section 2.3 was developed and LC–MS analysis was performed.

The mass spectra (Fig. 2) of mosapride and the polar impurity generated by LC–MS were compared. The mass spectrum of impurity displayed the protonated molecular ion at $m/z = 404$. The molecular mass of mosapride was observed at $m/$

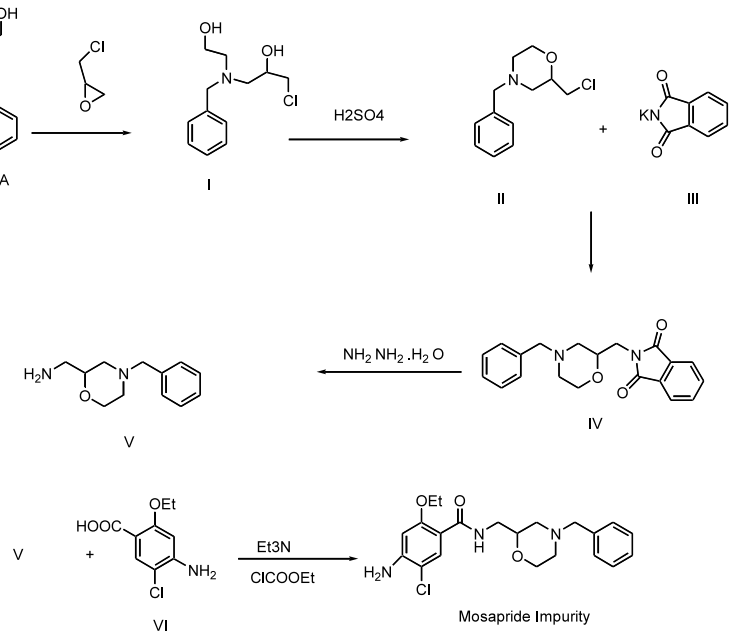


Fig. 6. Scheme for the synthesis of mosapride and impurity.

$z = 422$ which corresponds to the molecular formula $C_{21}H_{25}ClN_3O_3F$. The difference observed between these two is 18 amu.

To get structural information mass fragmental studies were taken up. In MS/MS data the fragments at $m/z = 198$ and 170 with characteristic chlorine isotopic abundance are identical in both mosapride and its impurity. This indicates that the 4-amino 5-chloro-2-ethoxy-benzamide moiety was presented in both the impurity and mosapride, whereas in non-chlorinated fragments, a diagnostic difference was observed. The mosapride MS/MS data displayed the daughter ion at $m/z = 109$, which corresponds to fluoro tropylium ion, whereas the impurity data showed a tropylium ion fragment only ($m/z = 91$) (Fig. 3). The fluoro tropylium ion and tropylium ion from the mosapride and its impurity are formed from the respective benzyl morphinyl moieties. This clearly indicated that a fluorinated benzyl group was present in mosapride and one benzyl group in the impurity. Based on this MS/MS data the desfluoro structure was proposed for mosapride. The fragmentation pathways based on the MS/MS data were displayed in Fig. 4.

3.2. Synthesis of impurity

The reaction of 2-benzyl amino ethanol (1A) with epichlorohydrin at 0–10 °C resulted in an intermediate diol (I), which was cyclised in acidic medium (H_2SO_4) at 70–80 °C to give 4-Benzyl-2-chloro methyl morpholine (II). The chloro group is replaced by phthalimido group by treating (II) with potassium phthalimide (III) in refluxing DMF to give (IV). The conversion of (IV) to (V) was effected by hydrazine hydrate and dilute HCl in alcoholic solvents. The condensation of (V) with 4-amino-5-chloro-2-ethoxy benzoic acid (VI) was carried out by using ethyl chloroformate and triethyl amine in dichloromethane to give mosapride impurity (Fig. 6) [4,5]. The synthesised impurity HPLC retention time matched well with the impurity present in pharma sample.

3.3. Structure elucidation of impurity

The spectral data of impurity was compared with mosapride. In IR spectrum C–F stretching was not observed like in mosapride at 1103 cm^{-1}. In mass spectrum the molecular ion was observed

Table 1
NMR assignments of mosapride and its impurity

Position[a]	Mosapride					Mosapride impurity					
	^{1}H	δ (ppm)	J (Hz)[b]	^{13}C	J (Hz)[c]	DEPT	^{1}H	δ (ppm)	J (Hz)[b]	^{13}C	DEPT
1	–	–	–	110.1	–	–	–	–	–	110.9	–
2	–	–	–	156.8	–	–	–	–	–	156.9	–
3	1H	6.50	s	98.4	–	CH	1H	6.27	s	98.3	CH
4	–	–	–	148.5	–	–	–	–	–	146.8	–
$4NH_2$	2H[c]	4.20	s	–	–	–	2H[c]	4.37	s	–	–
5	–	–	–	109.2	–	–	–	–	–	111.8	–
6	1H	7.70	–	131.6	–	CH	1H	8.11	–	132.5	CH
7	–	–	–	163.7	–	–	–	–	–	164.5	–
8	NH[c]	8.10	br	–	–	–	NH[c]	8.21	br	–	–
9	Ha	3.50	–	41.5	–	CH_2	Ha	3.71	–	42.0	CH_2
	Hb	3.26					Hb	3.36			
10	1H	3.60	–	73.6		CH	1H	3.71	–	74.3	CH
11	He	2.90	d,11.6	54.9		CH_2	He	2.80	d,11.6	55.8	CH_2
	Ha	2.20	t, 10.8				Ha	2.02	t, 10.8		
13	He	2.70	d, 11.6	52.0		CH_2	He	2.68	d, 11.6	52.7	CH_2
	Ha	2.20	ddd, 10.8,				Ha	2.20	ddd, 10.8,		
14	He	3.88	d, 11.2	64.6		CH_2	He	3.88	d, 11.2	66.5	CH_2
	Ha	3.60	–				Ha	3.74	–		
15	Ha	3.44	d, 13.2	60.9		CH_2	Ha	3.54	d, 13.2	64.6	CH_2
	Hb	3.30	d, 13.2				Hb	3.50	d, 13.2		
16	–	–	–	132.4		–	–	–	–	137.3	–
17	1H	7.2–7.4	–	131.3	8.2	CH	1H	7.2–7.4	–	128.1	CH
18	1H	7.2–7.4	–	115.0	21.0	CH	1H	7.2–7.4	–	128.9	CH
19	–	–	–	161.6	245.0	CH	1H	7.2–7.4	–	127.0	CH
20	1H	7.2–7.4	–	115.1	21.0	CH	1H	7.2–7.4	–	128.9	CH
21	1H	7.2–7.4	–	131.3	8.2	CH	1H	7.2–7.4	–	128.1	CH
22	2H	4.08	q, 7.0	65.4		CH_2	2H	4.08	q, 7.2	63.0	CH_2
23	3H	1.50	t, 7.0	14.5		CH_3	3H	1.50	t, 7.2	14.5	CH_3

[a] Refer the structural formula in Fig. 5 for numbering.
[b] $^{1}H-^{1}H$ coupling constants.
[c] $^{19}F-^{13}C$ coupling constants.

at 18 amu less than mosapride. In ^{1}H and ^{13}C NMR the couplings due to fluorine was not observed. In DEPT additional aromatic CH was observed in impurity. These observations confirmed the molecular structure of impurity as 4-amino-5-chloro-2-ethoxy-N-[[(4-benzyl)-2-morphinyl]methyl]benzamide.

The NMR assignments were listed in the Table 1.

3.4. Formation of impurity

Formation of this impurity was rationalised by the presence of 2-[(4-benzyl) amino] ethanol as impurity in 2-[(4-fluorobenzyl) amino] ethanol, which is the starting compound in the synthesis of mosapride. It is condensing with chloro methyl oxirane followed by cyclisation to give 2-chloro, 4-benzyl morpholine, which leads to impurity as in Scheme-I

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