Department of Analytical Research, Discovery Research[1], Dr. Reddy's Laboratories Ltd., Miyapur, Hyderabad; Dr. Reddy's Holdings Pvt. Ltd.[2], Dr. Reddy's Laboratories Ltd., Hyderabad, India

Structural studies of racecadotril and its process impurities by NMR and mass spectroscopy

K. Mallikarjun Reddy[1], J. Moses Babu[1], P. Sudhakar[1], M. S. P. Sharma[2], G. Sudershan Reddy[2], K. Vyas[1]

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Dr. J. Moses Babu, Department of Analytical Research, Discovery Research, Dr. Reddy's Laboratories Ltd., Bollaram Road, Miyapur, Hyderabad 500050, India
mosesbabuj@drreddys.com

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Three unknown impurities in racecadotril bulk drug at levels below 0.5% were detected by simple reverse phase isocratic high performance liquid chromatography (HPLC). Structures for these impurities were proposed by molecular ion information and their fragmentation pattern obtained by LC-MS and these impurities were confirmed by NMR spectroscopy. The impurities I, II and III were characterized as benzyl 2-methyl carboximido acetate, benzyl 2-phenyl ethyl carboximido acetate, and benzyl 2-(1-benzyl vinyl carboximido) acetate. These structures were further confirmed by co-injecting of synthetic standards of impurities with racecadotril. The mechanism of the formation of these process related impurities is discussed.

1. Introduction

Racecadotril, *N*-[(*R*,*S*)-3-(acetylmercapto)-2-benzyl propanoyl] glycine benzyl ester, is an antidiarrheal drug inhibiting enkephalinase (Alam et al. 2003). Studies have shown that racecadotril inhibits intestinal secretion induced by chemical and microbiological agents without reducing gastrointestinal transit time (Schwartz et al. 2000). It is an effective and safe treatment for acute diarrhea in adults and children.

During the analysis of racecadotril bulk drug three impurities which were present in the level $\leq$0.5% were detected. The impurities at levels below 0.05% must be identified and characterized in the bulk drug. This study aims at the characterization of three major impurities I, II and III in racecadotril using LC-MS (Kumar et al. 2003) and NMR (Sattanathan et al. 2005).

2. Investigations, results and discussion

2.1. Detection of impurities by LC-MS

The LC-MS method described in section 3.2 was used to detect the impurities (Fig. 1). Three polar impurities were detected at 3.27 min, 5.98 min and 7.09 min RT with respect to racecadotril RT 7.93 min (Table 1). Based on molecular ion information structures of the impurities were proposed.

2.2. Structure elucidation of impurities

2.2.1. Impurity-I

The positive ES-MS spectrum (Fig. 2) of impurity-I showed peaks at m/z 208.1, 230.3 and 246.1 corresponding to $(M + H)^+$, $(M + Na)^+$ and $(M + K)^+$. The molecular ion of impurity is 178 mass units less than that of racecadotril (Table 1). The ^{1}H NMR spectrum of impurity-I displayed a methyl, two methylene and five aromatic methine resonances at δ 2.0, 4.1, 5.2 and 7.1–7.4 ppm respectively. The ^{13}C NMR spectrum of impurity-I showed ten carbon atoms less compared to that of racecadotril. Two characteristic carbonyl carbon signals were found at 169.5 and 170.6 in the impurity. The C-S stretching absorption band found in FT-IR spectrum of

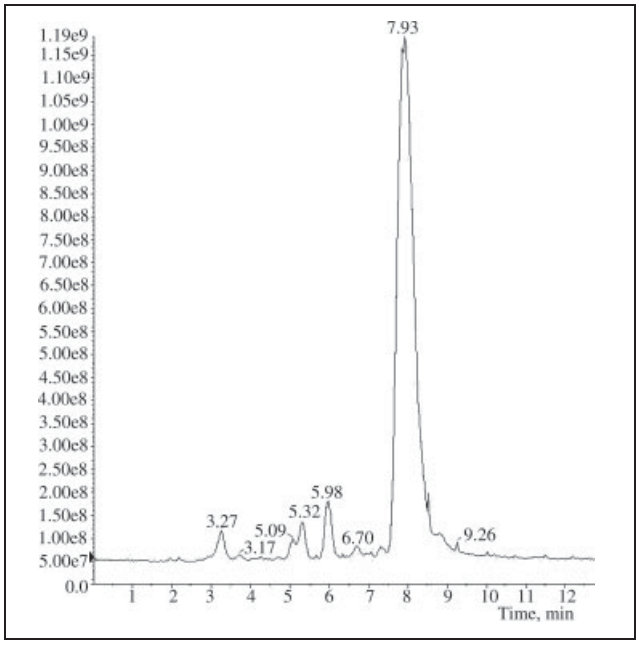
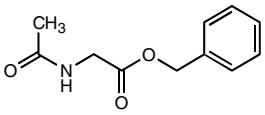
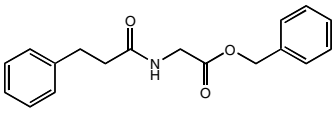
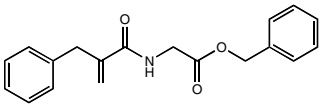
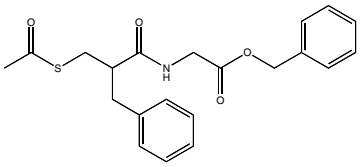


Fig. 1: LC-MS Analysis of racecadotril RDC-4008

Table 1: LC-MS information of the racecadotril, impurities I, II and III

Tentative structure	Retention time (min)	$(M + H)^+$
 Impurity-I	3.27	208.1
 Impurity-II	5.98	298.0
 Impurity-III	7.09	310.0
 Racecadotril	7.93	386.0

racecadotril at 625 cm^{-1} was absent in the impurity. Based on the above spectral data, the molecular formula of impurity I could be $C_{11}H_{13}NO_3$. This molecular formula matches well with the protonated molecular ion observed at m/z 208.1 in the MS. This evidence and the complete assignments of the ^1H and ^{13}C NMR resonances confirm the structure of impurity-I as benzyl 2-methyl carboximido acetate.

2.2.2. *Impurity-II*

The spectral data of impurity-II were compared with that of impurity-I. The positive ES-MS (Fig. 3) of impurity-II displayed peaks at m/z 298.0, 320.0 and 336.0 corresponding to $(M + H)^+$, $(M + Na)^+$ and $(M + K)^+$. The molecular ion of impurity-II is 90 atomic mass units more than that of impurity-I (Table 1). This indicates the possibility of the incorporation of a benzyl moiety in impurity-I. The ^1H NMR spectrum of impurity-II displayed two methylene signals and five aromatic methine signals at δ 2.9, 2.5 and 7.1–7.4 ppm respectively in excess to impurity-I. The methyl signal in impurity-I at δ 2.0 ppm and δ 22.1 ppm in ^1H NMR and ^{13}C NMR spectra were not observed in impurity II, instead a signal at δ 2.5 ppm in ^1H NMR spectrum corresponding to methylene protons were found. In the ^{13}C NMR spectrum two methylene and five aromatic methine signals appeared in excess in comparison with that of impurity I. From the above spectral data, the molecular formula of impurity II could be $C_{18}H_{19}NO_3$. This molecular formula matches well with the protonated molecular ion observed at m/z 298.1 in the MS. The structure of impurity-II has been characterized as benzyl 2-phenyl ethyl carboximido acetate.

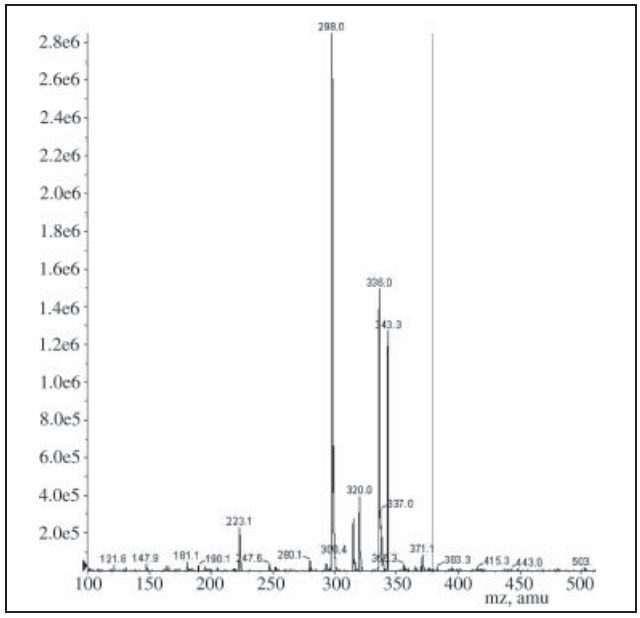


Fig. 3: Positive ES-MS spectrum of racecadotril impurity-II

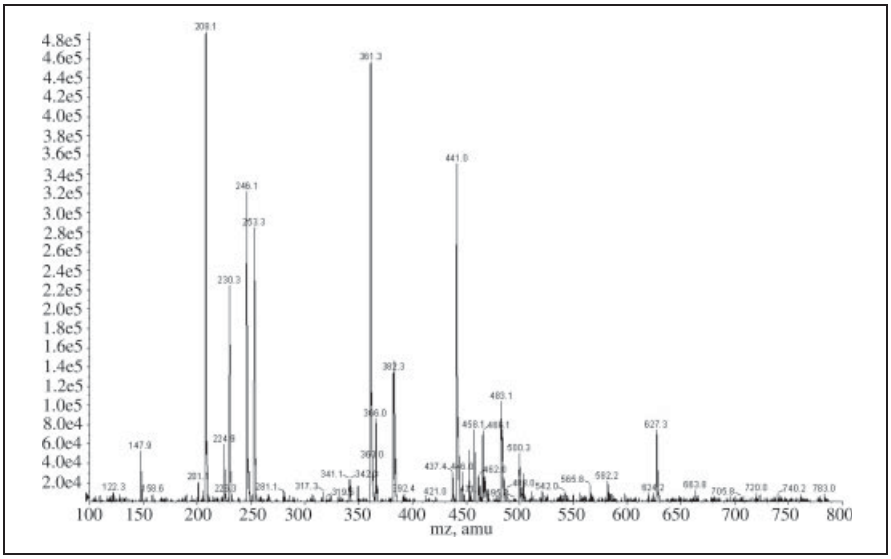


Fig. 2:
Positive ES-MS spectrum of racecadotril impurity-I

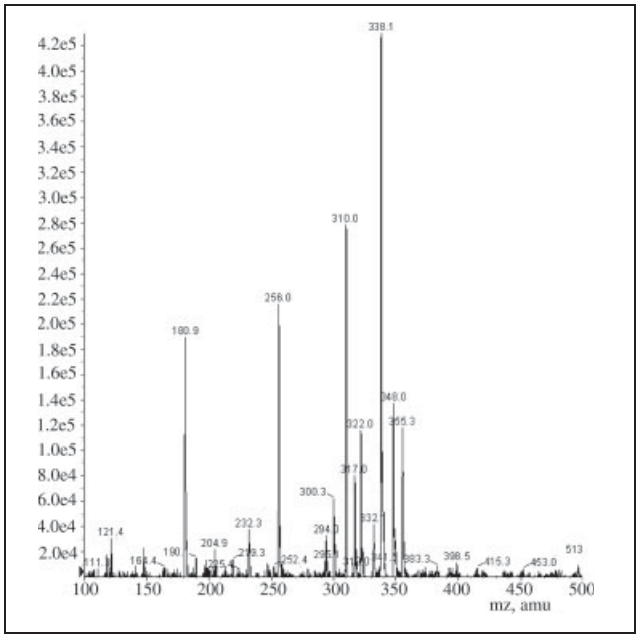


Fig. 4: Mass spectrum of racecadotril impurity-III

2.2.3. Impurity-III

The positive ES-MS spectrum (Fig. 4) of impurity-III displayed peaks at m/z 310.0, 322.0 and 338.1 corresponding to $(M + H)^+$, $(M + Na)^+$ and $(M + K)^+$. The molecular ion of impurity-III is 12 atomic mass units more than that of impurity-II (Table 1). The ^{1}H NMR spectrum of impurity-III displayed characteristic vinylic proton signals at δ 5.8, 5.3 ppm. The methylene proton signal at δ 2.5 ppm in impurity-II was absent in impurity III. The ^{13}C NMR spectrum of impurity-III displayed additional quaternary carbon δ 143.5 ppm and characteristic vinylic carbon at δ 120.6 ppm when compared to that of impurity-II. The above data can be rationalized in terms of incorporation of a vinyl group. Based on the above spectral data, the molecular formula of impurity III could be $C_{19}H_{19}NO_3$. The structure of impurity-III has been characterized as benzyl 2-(1-benzyl vinyl carboximido) acetate.

2.3. Formation of impurities

Impurity I was formed when traces of acetyl chloride reacts with benzylglycinate which is a reactant used in the

Scheme

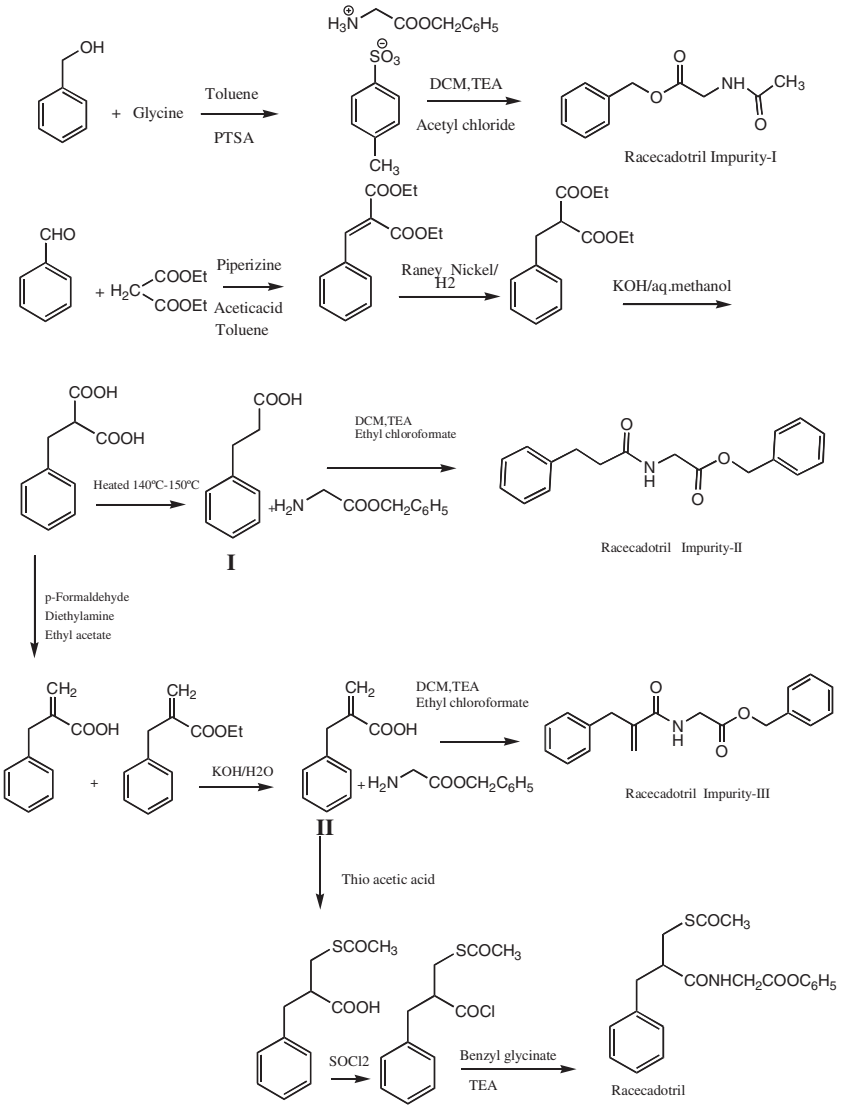


Table 2: FT-IR frequency assignments for racecadotril, impurities I, II and III

Mode of vibration	Racecadotril	Impurity-I	Impurity-II	Impurity-III
NH*	3289	3296	3320	3343
Aromatic C−H*	3085	3068	3030	3030
Aliphatic C−H*	2933	2939	2924	2936
C=O/Amide*	1732, 1688/1643	1749/1660	1743/1643	1748/1659
C=C*	1550	1546	1551	1620, 1528
C−O*	1293, 1134	1191	1211	1187
Aromatic C−H*	694	699	699	699
C−S*	625	–	–	–

Table 3: NMR assignments for the racecadotril, and the impurities I, II and III

Position[a]	Racecadotril			Impurity-I			Impurity-II			Impurity-III		
	^{1}H	ppm/J	^{13}C	^{1}H	ppm/J	^{13}C	^{1}H	ppm/J	^{13}C	^{1}H	ppm/J	^{13}C
1, 5	2 H	7.1–7.4/m	126.4	2 H	7.1–7.4/m	127.7	2 H	7.1–7.4/m	127.9	2 H	7.1–7.4/m	128.2
2, 3, 4	3 H	7.1–7.4/m	128.3	3 H	7.1–7.4/m	127.9	3 H	7.1–7.4/m	128.2	3 H	7.1–7.4/m	128.4
6	–	–	135.0	–	–	134.9	–	–	135.0	–	–	135.0
7	2 H	5.1/s	66.9	2 H	5.2/s	66.4	2 H	5.2/s	66.7	2 H	5.2/s	67.1
8	–	–	169.2	–	–	169.5	–	–	169.6	–	–	169.0
9	2 H	4.1/dd (4.9, 18.2)	41.2	2 H	4.1/d (10.4)	40.9	2 H	4.1/d (9.6)	41.1	2 H	4.1/d (10.0)	41.5
10	NH	5.9/br	–	NH	6.3/br	–	NH	5.9/br	–	NH	6.3/br	–
11	–	–	172.9	–	–	170.6	–	–	172.4	–	–	169.6
12	1 H	2.6/m	48.9	3 H	2.0/s	22.1	2 H	2.5/t (14.2)	37.3	–	–	143.5
13	2 H	2.9/m	38.2	–	–	–	2 H	2.9/t (14.2)	31.1	2 H	3.7/s	38.3
14	–	–	138.4	–	–	–	–	–	140.5	–	–	138.0
15, 19	2 H	7.1–7.4/m	128.7	–	–	–	2 H	7.1–7.4	128.3	2 H	7.1–7.4/m	128.6
16, 18	2 H	7.1–7.4/m	128.1	–	–	–	2 H	7.1–7.4	128.2	2 H	7.1–7.4/m	128.8
17	1 H	7.1–7.4/m	128.4	–	–	–	1 H	7.1–7.4	125.9	1 H	7.1–7.4/m	126.5
20	2 H	3.1/m	30.4	–	–	–	–	–	–	Ha Hb	5.8/s 5.3/s	120.6
22	–	–	195.7	–	–	–	–	–	–	–	–	–
23	3 H	2.3/s	31.0	–	–	–	–	–	–	–	–	–

a Refer the structural formula for numbering
J-Coupling constant, s-singlet, d-doublet, dd-doublet of doublet, t-triplet, m-multiplet, br-broad

synthesis. The intermediates I and II (Scheme) of rececadotril, react with benzyl glycinate resulting in the formation of impurity II and impurity III respectively.

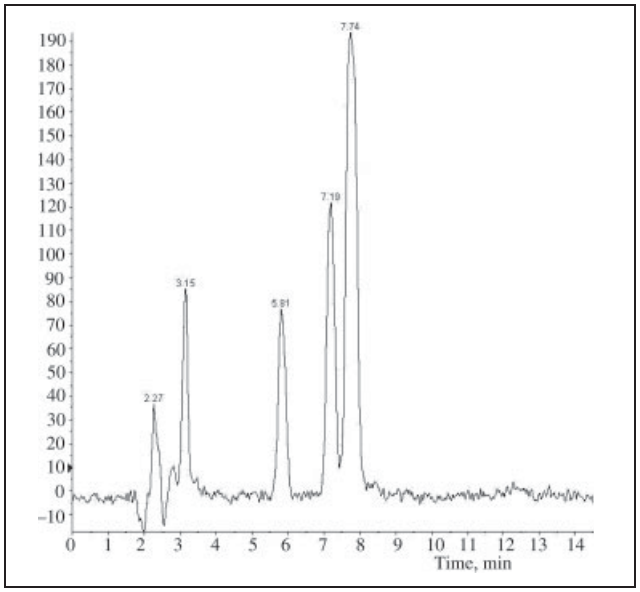


Fig. 5: Spiked chromatogram of racecadotril with impurities I, II and III

2.4. Conclusion

Characteristic FT-IR absorption frequencies recorded for racecadotril, Impurities I, II and III are given in Table 2. The ^{1}H and ^{13}C NMR assignments were listed in Table 3. The synthetic standards of the impurities prepared were co injected with racecadotril sample and their RRT's matches well in the LC-MS study (Fig. 5).

3. Experimental

3.1. Samples

The investigated samples of racecadotril (B. No.: RDC-4008) were obtained from Dr. Reddy's Holdings Ltd., Hyderabad, India. The materials used for LC-MS analysis were ammonium acetate (AR grade, SD fine chemicals, India), acetic acid (excela®, Qualigens) and acetonitrile (Gradient grade, Ranbaxy Laboratories, India). Water used was purified using Milli-Q plus purification system.

3.2. LC-MS study

An Agilent Model 1100 series module equipped with photodiode array UV detector coupled to triple quadrupole mass spectrometer PE Sciex model API 3000 was used. Symmetry shield C18 ODS 250 × 4.6 mm, 5 µ was used for the separations. The column eluent was monitored at a wavelength of 220 nm. Mass and DAD data were recorded using Analyst 1.3 software. A mixture of 0.01 M ammonium acetate (pH = 4.2 adjusted with acetic acid) and acetonitrile in the ratio 40 : 60 (v/v) was used as mobile phase at the flow rate 1.0 ml/min. The positive and negative electrospray ionization studies were performed by switching the capillary voltage be-

tween +5000 and −4500 V respectively (declustering potential +70v, and Focusing potential +180v).

3.3. Mass spectral study

The electrospray ionization studies were performed on a triple quadrupole mass spectrometer PE Sciex model API 3000. The positive and negative electrospray MS data was obtained by switching the capillary voltage between +5000 and −4500 V. The MS–MS data was generated with the collision energy ramping from 30 to 60 V in nitrogen atmosphere.

3.4. FT-IR study

The FT-IR spectra of racecadotril, impurities I, II and III were recorded in the solid state as KBr dispersion Perkin-Elmer 1600 FT-IR spectrophotometer.

3.5. NMR study

The ^{1}H and ^{13}C NMR experiments of racecadotril, impurities I, II and III were performed in $CDCl_3$ using Varian Mercury plus 400 MHz and Varian Gemini 200 MHz NMR instruments, respectively. ^{1}H and ^{13}C chemical shifts are reported on the δ scale in ppm relative to TMS (δ 0.00) and $CDCl_3$ (δ 77.00) as internal standards respectively.

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References

Alam NH, Asharaf H, Khan WA, Karim MM, Fuchs GJ (2003) Efficacy and tolerability of racecadotril in the treatment of cholera in adults. Gut 52: 1419–1423.

Ravindra Kumar Y, Moses Babu J, Sarma MSP, Seshidhar B, Srinivasa Reddy S, Sudarsan Reddy G, Vyas K (2003) Application of LC-MS/MS for the identification of a polar impurity in mosapride, a gastroprokinetic drug. J Pharm Biochem Anal 32: 361–368.

Sattanathan P, Moses Babu J, Vyas K, Reddy RB, Rajan ST, Sudhakar P (2006) Structural studies of impurities of risperidone by hyphenated techniques. J Pharm Biochem Anal 40: 598–604.

Schwartz JC (2000) Racecadotril: A new approach to the treatment of diarrhoea. Int J Antimicrob Agents 14: 75–79.