



RESEARCH ARTICLE

CHARACTERIZATION AND IDENTIFICATION OF TIAPRIDE HYDROCHLORIDE RELATED SUBSTANCE IMPURITIES BY HPLC, LCMS AND NMR TECHNIQUES

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Received on: 1st May 2025, Accepted on 1st July 2025, Published on: 26th August 2025

ABSTRACT

Tiapride hydrochloride, an antipsychotic agent widely prescribed for the treatment of schizophrenia and various neurological disorders, was subjected to impurity profiling using advanced analytical techniques, including HPLC, LC-MS, and NMR spectroscopy. A stability-indicating LC-MS method was successfully developed and validated for the quantitative determination of tiapride in its tablet dosage form. Forced degradation studies were conducted under hydrolytic, oxidative, thermal, and photolytic stress conditions to evaluate the drug's stability. These studies led to the formation of three degradation products (DP1, DP2, and DP3), which were subsequently isolated using preparative HPLC. Structural elucidation of the impurities was performed using LC-MS, one-dimensional NMR (¹H and ¹³C) techniques. The identified degradation products were: DP1 – N-(2-(ethylamino) ethyl)-2-methoxy-5-(methylsulfonyl) benzamide, DP2 – 2-methoxy-5-sulfonylbenzoic acid, DP3 – N-[2-(diethylnitroxyl) ethyl]-2-methoxy-5-(methylsulfonyl)benzamide (tiapride N-oxide). The validated LC-MS method exhibited excellent selectivity, specificity, accuracy, precision, and robustness for impurity detection in tiapride formulations. This comprehensive study provides critical insights into the

degradation behavior of tiapride and contributes to ensuring the long-term safety and quality of both the active pharmaceutical ingredient (API) and its finished dosage forms. These findings serve as a valuable reference for future formulation development and stability studies of Tiapride-containing products.

Keywords: *Tiapride Hydrochloride, Degradation Products, Forced Degradation Studies, HPLC, LC/MS, NMR Spectroscopy, Related Substance, Impurities Profiling.*

INTRODUCTION

Tiapride hydrochloride, chemically known as N-(2-diethylaminoethyl)-2-methoxy-5-alkylsulfonylbenzamide, is classified as an atypical neuroleptic agent and is primarily indicated for the treatment of schizophrenia [1] (Figure 1). Its therapeutic efficacy arises from its selective antagonism of dopamine D₂ receptors, particularly within the limbic system and the locus coeruleus. By binding to these dopaminergic receptors, tiapride effectively inhibits their activity and

suppresses the release of associated neurotransmitters.

This modulation of dopaminergic signaling within the limbic region plays a key role in alleviating the positive symptoms of schizophrenia. Beyond its antipsychotic activity, tiapride is also used in the management of other neurological and psychiatric conditions, including Huntington's disease, Tourette's syndrome, chronic intractable pain, and alcohol dependency [2–8].

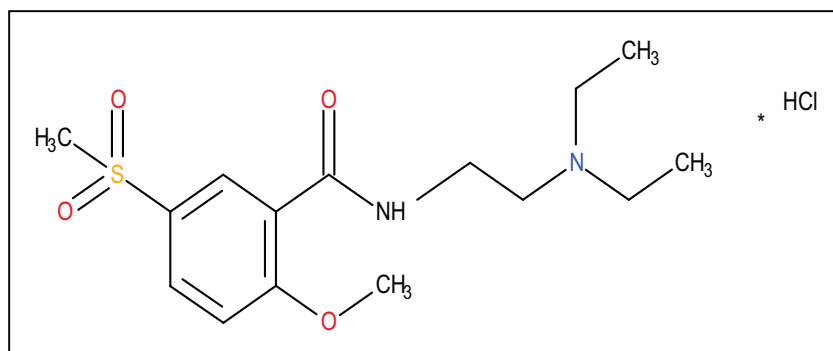


Figure 1: Structure of Tiapride Hydrochloride

Numerous analytical methodologies have been reported for the quantification of tiapride in biological samples. These include liquid chromatography techniques coupled with spectrofluorimetric detection [9], UV spectrophotometry [10],

photodiode array detection, and mass spectrometry-based approaches for determining tiapride levels in biological matrices.

In addition to these, several alternative techniques—such as polarimetry, high-performance thin-layer chromatography (HPTLC) [11], gas chromatography (GC) [12], and hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS) [13] have also been employed to analyze tiapride under diverse conditions.

An isocratic high-performance liquid chromatography (HPLC) method for the profiling of degradation impurities A–E in the active pharmaceutical ingredient (API) tiapride is documented in the Japanese Pharmacopeia (JP) [14, 15]. Capillary zone electrophoresis (CZE) and voltametric techniques have also been applied to separate tiapride hydrochloride and its related substances in both bulk pharmaceutical forms and biological fluids such as human plasma and urine.

Furthermore, the European Pharmacopeia and the British Pharmacopeia list a related substances RP-HPLC method for the analysis of tiapride hydrochloride API. However, despite these advancements, a universally applicable method specifically optimized for tablet formulations is still lacking. This presents a significant analytical challenge—particularly for LC-MS-based techniques—in detecting low-level impurities within complex pharmaceutical excipient matrices [16–18].

Forced degradation is a purposeful process in which pharmaceutical substances or products are exposed to more severe

conditions than those used in accelerated stability studies. The objective is to induce the formation of degradation products that help in identifying potential impurities that could arise during manufacturing, storage, or usage. These degradation products often differ significantly in chemical structure from the parent compound, thus providing valuable insights into the drug's stability profile.

Strict impurity limits for APIs and finished dosage forms have been established by the British Pharmacopoeia and the United States Pharmacopeia [19–21]. Conducting degradation studies early in the drug development process is strongly recommended to ensure the timely generation of comprehensive stability data. Regulatory guidelines mandate the identification and structural characterization of degradation products that exceed specific thresholds [22–24]. Such studies play a critical role in the development of stability-indicating analytical methods and provide essential data to ensure drug safety and efficacy over its shelf life [25–27].

A study was conducted to develop and validate a reverse-phase high-performance liquid chromatography (RP-HPLC) method for quantifying tiapride-related impurities in tablet formulations [28]. During forced degradation experiments, three novel impurities—previously undocumented in the literature—were detected and effectively separated under optimized chromatographic conditions.

Given the absence of prior reports on these degradation products, detailed structural elucidation became essential. Consequently, the present research focused on the identification and characterization of these unknown impurities using advanced analytical techniques, with particular emphasis on liquid chromatography-mass spectrometry (LCMS) and nuclear magnetic resonance (NMR) spectroscopy.

This study reports the outcomes of related substance analysis for tiapride drug substance stored under stress conditions of 40 °C and 75% relative humidity (RH). In accordance with ICH guidelines, impurities that exceed identification thresholds must be structurally characterized using appropriate analytical tools. In the present investigation, both known and unknown degradation products detected during storage were analyzed using LC-MS. To overcome the challenge of identifying impurities present in trace quantities, larger amounts were deliberately generated via alkaline and oxidative forced degradation. These products were then isolated using preparative liquid chromatography, followed by comprehensive structural characterization using spectroscopic techniques.

MATERIALS AND METHODS

Chemical and Reagents

All chemicals and reagents used in this study were obtained from reputable

sources. Methanol (HPLC grade) and hydrochloric acid were supplied by Merck Specialities Pvt. Ltd., Mumbai. Purified water was generated using a Pure diamond water purification system. Analytical reagent (AR) grade sodium hydroxide, hydrogen peroxide, and potassium dihydrogen orthophosphate were procured from Rankem Ltd. Sodium octane sulfonate (AR grade) and orthophosphoric acid (HPLC grade) were sourced from Spectrochem Ltd. The working standard of tiapride hydrochloride was provided by easy buyer (Hong Kong) Ltd., while the commercial tiapride hydrochloride tablet formulation was obtained from INTAS Pharmaceuticals Ltd., Ahmedabad, Gujarat, India.

INSTRUMENTATION

Liquid Chromatography system and Method

High-performance liquid chromatography (HPLC) analysis was conducted using an Agilent 1100/1200 series system, equipped with a Diode Array Detector and a quaternary pump, and operated through Chromeleon software. pH measurements were taken using a PHAN meter from Lab India Ltd., offering a precision of ± 0.01 pH units. The instrument employed a glass-calomel electrode calibrated with standard buffer solutions at pH values of 4.0, 7.0, and 9.0. The mobile phase consisted of a mixture of phosphate buffer, acetonitrile, and methanol in a volumetric ratio of 800:150:50 (v/v/v). The phosphate

buffer (0.05 M, pH 2.7) was filtered through a 0.45 μm nylon membrane and degassed by sonication for 15 minutes. Chromatographic separation was achieved on an Inertsil C8 column (250 $\times$ 4.6 mm, 5 μm particle size) maintained at 40 $^{\circ}\text{C}$, with a flow rate of 1.5 mL/min, an injection volume of 10 μL , and UV detection set at 240 nm [28].

Preparative Liquid Chromatography

For preparative liquid chromatography, the isolation of an unknown impurity was performed using an Agilent 1100/1200 series system, equipped with a Diode Array Detector and a quaternary pump, with automated fraction collection controlled via Chromeleon software. pH measurements were carried out using a PHAN meter from Lab India Ltd., featuring an accuracy of ± 0.01 pH units. The instrument was fitted with a combined glass–calomel electrode and calibrated using standard buffer solutions at pH values of 4.0, 7.0, and 9.0. Prior to use, the mobile phase composition—phosphate buffer, acetonitrile, and methanol in an 800:150:50 v/v/v ratio—was filtered through a 0.45 μm nylon membrane and degassed using 15 minutes of sonication. Preparative HPLC was conducted on a C18 OBD Prep Column (30 $\times$ 100 mm, 5 μm , 125 $\text{\AA}$) at a flow rate of 20 mL/min, with UV detection set at 240 nm. The isolated impurity fractions were then concentrated by solvent evaporation under high vacuum using a Rotavapor VR R-215 (BuchiVR

Labortechnik, Switzerland) rotary evaporator [28].

Liquid Chromatography Mass Spectrometry (LCMS)

Liquid chromatography–mass spectrometry (LC-MS) analysis was conducted using an Agilent 6310 ion trap LC-MS system, controlled via Trap Control software. The mobile phase employed a gradient program comprising mobile phase A (0.1% formic acid in water) and mobile phase B (HPLC-grade acetonitrile), both of which were degassed by sonication for 5 minutes before use. Chromatographic separation took place on an Inertsil C8 column (250 $\times$ 4.6 mm, 5 μm ; GL Sciences) maintained at 40 $^{\circ}\text{C}$. The flow rate was set at 1.5 mL/min with an injection volume of 10 μL . UV detection was performed at 240 nm, while mass spectrometric analysis was carried out using the ion trap mass analyzer [28].

Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear magnetic resonance (NMR) spectra for both ^1H and ^{13}C nuclei were recorded on a Bruker Advance II spectrometer (Bruker, Fallanden, Switzerland) at 290.5 K. Deuterated dimethyl sulfoxide (DMSO- d_6) was used as the solvent and internal reference. The ^1H NMR spectra were acquired at a frequency of 400 MHz, while the ^{13}C spectra were obtained at 100 MHz. NMR analysis was conducted to enable precise proton chemical shift assignments and to

support comprehensive structural characterization of the compound.

Forced Degradation Studies

The degradation study of tiapride hydrochloride was conducted under various stress conditions to assess its stability, including hydrolysis, oxidation, thermal degradation, and photolysis, in accordance with ICH guidelines. A stock solution of tiapride at 1000 µg/mL was prepared using the mobile phase as a diluent. For hydrolytic degradation, eight accurately weighed intact tablets were dispersed in 150 mL of diluent in a 200 mL volumetric flask and sonicated for 15 minutes. Acidic hydrolysis was performed by adding 8 mL of 5 M hydrochloric acid, followed by refluxing at 80 °C for 24 hours and neutralization with 8 mL of 5 M sodium hydroxide. Similarly, basic hydrolysis was carried out by adding 8 mL of 5 M sodium hydroxide, refluxing at 80 °C for 24 hours, and neutralizing with 8 mL of 5 M hydrochloric acid. Neutral hydrolysis involved the addition of 8 mL of distilled water and refluxing at 80 °C for 24 hours. Oxidative stress was induced by treating the sonicated drug mixture with 8 mL of 30% hydrogen peroxide and refluxing at 80 °C for 24 hours. After each hydrolysis or oxidation study, the solutions were allowed to cool to room temperature, the volume was adjusted to 200 mL with diluent, and the samples were filtered through a 0.45 µm nylon filter. Thermal degradation was studied by maintaining both solid drug samples and 1% aqueous

solutions in petri dishes inside a stability chamber at 105 °C and 75% relative humidity for 48 hours. Photolytic degradation was performed by exposing the solid drug, placebo powder, and 1% aqueous solution to light in a UV chamber for a minimum of 72 hours, ensuring overall illumination as per ICH Q1B guidelines. Placebo and blank solutions were prepared in a similar manner but without the active pharmaceutical ingredient. All forced degradation samples were analyzed after dilution and filtration, ensuring consistency in reagent volumes, exposure conditions, and treatment durations to allow for accurate comparative degradation profiling [28].

Sample Preparation

Eight whole tablets, each containing 25 mg of tiapride API (totalling 200 mg), were accurately weighed and transferred into a 200 mL volumetric flask. Then, 150 mL of diluent was added, and the mixture was sonicated for 15 minutes to disperse the tablets completely while stirring. The resulting solution was filtered through a 0.45 µm nylon membrane filter prior to analysis.

Isolation of Degradation Product

To isolate degradation products generated under alkaline and oxidative stress, the solutions from the forced degradation experiments were concentrated by removing the solvent under high vacuum using a rotary evaporator. The concentrated samples were then loaded onto a

preparative HPLC column to collect pure fractions of individual degradation products. These fractions were subsequently analyzed by analytical HPLC to verify their relative retention times (RRT) and determine their purity, with each degradation product showing purity levels above 95.0%. After purification, methanol was evaporated using a rotary evaporator and a water bath, yielding the degradation products as solid residues. Specifically, degradation products DP1, DP2, and DP3 were isolated from the evaporation of the chloroform layer. These solid impurities were then subjected to further characterization using a range of spectroscopic methods including Liquid Chromatography-mass spectroscopy and nuclear magnetic resonance (^1H and ^{13}C NMR).

RESULT AND DISCUSSION

Liquid Chromatography-Mass Spectrometry

Although tiapride is known to exhibit high stability, it was observed to undergo significant degradation under forced stress conditions. While several analytical methods for analysing tiapride API are

documented in pharmacopoeia, no established method existed for the characterization of related substances in the solid dosage form. Notably, hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry (HILIC-MS/MS) has been used to determine tiapride concentrations in human plasma. However, a robust, stability-indicating LC-MS method for tablet formulations is unavailable.

The aim of this study was to develop a simple and reliable LC-MS method to detect degradation products in tablet form. The method was optimized using an Inertsil C8 column (250×4.6 mm, $5\ \mu\text{m}$), operated with a gradient elution program. The mobile phase consisted of 0.1% formic acid in water (Mobile Phase A) and HPLC-grade acetonitrile (Mobile Phase B). The flow rate was maintained at 1.5 mL/min, and the column temperature was set at $40\ ^\circ\text{C}$.

This chromatographic configuration offered optimal resolution and symmetrical peak shapes for tiapride and its related substances. The finalized gradient program is detailed in Table 1.

Table 1: Gradient Programme

Time (min)	Mobile Phase A (% v/v)	Mobile Phase B (% v/v)
00	95	05
05	95	05
15	60	40
20	60	40
21	95	05
25	95	05

The results confirmed that neither the blank nor the placebo samples exhibited any peaks at the retention time corresponding to that of tiapride, indicating the specificity of the method. Tiapride hydrochloride tablets demonstrated significant degradation when subjected to

oxidative stress (Figure 2), as well as under alkaline and acidic hydrolytic conditions. In all cases, the resulting degradation products were well resolved from the parent tiapride peak, confirming the method's ability to separate impurities effectively.

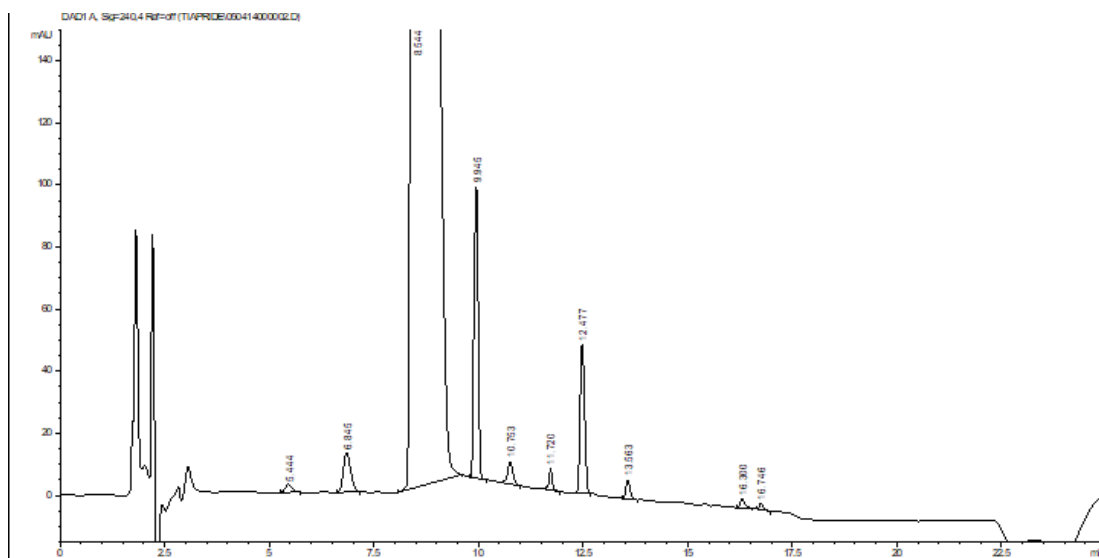


Figure 2: Chromatogram of Oxidative stress Condition

In contrast, no notable degradation was observed under neutral hydrolytic (water) or thermal stress conditions. Importantly, across all related substance analyses of the degradation samples, the tiapride peak remained spectrally pure and consistent, further validating the selectivity and stability-indicating nature of the developed LC-MS method.

Detection and Quantification of Degradation Products by HPLC

A chromatographic method was developed and validated to achieve adequate resolution for impurity profiling. Under basic and oxidative stress conditions, three degradation products DP1, DP2, and DP3 were detected, contributing to

approximately 7.0% total degradation. The combined level of individual impurities reached up to 1.14%, exceeding the ICH identification threshold of 0.05%. Consequently, these degradation products were selected for further isolation and structural characterization using a combination of spectroscopic and spectrometric techniques.

Due to their initially low abundance in stressed samples, additional forced degradation was carried out to obtain sufficient quantities of each impurity for isolation. Among all tested stress conditions, oxidative degradation proved to be the most significant contributor to the formation of DP1, DP2, and DP3 (Table 2).

Table 2: Force Degradation Study of Tiapride Hydrochloride

Condition	Related Impurity		Peak Purity Match Factor	% Assay of Tiapride	% Degradation
	% Single Impurity	% Total Impurity			
Without Degradation (Control Condition)	0.046	0.066	997.711	99.9	0.00
Hydrolytic Degradation (Acid) 5 M HCL/ 80° C / 72 hr	4.322	5.379	998.201	94.1	5.80
Hydrolytic Degradation (Alkali/basic) 5 M NaOH/ 80°C / 72 hr	5.293	6.766	998.334	92.9	7.00

Oxidative Degradation 80°C/ 30% H₂O₂	6.097	7.323	998.426	91.6	7.00
Thermal Degradation (105° C)	0.058	0.148	997.803	99.2	0.70
Hydrolytic Condition (Water) 80° C/ 72 hr	0.046	0.093	997.864	99.4	Not Detected
Photo Degradation (Uncontrolled)	0.049	0.068	997.938	99.5	Not Detected
Photo Degradation (Uncontrolled)	0.045	0.064	997.803	99.6	Not Detected

Isolation of DP1, DP2, DP3

The degradation behavior of tiapride was systematically studied under oxidative and alkaline stress conditions. Oxidative degradation using hydrogen peroxide led to the formation and isolation of DP1, DP2, and DP3. In parallel, alkaline hydrolysis was employed to produce a

higher yield of degraded tiapride for further analysis.

Crude samples obtained through preparative liquid chromatography (HPLC) exhibited a purity level exceeding 95% for each degradation product. These were subsequently characterized using advanced spectroscopic methods.

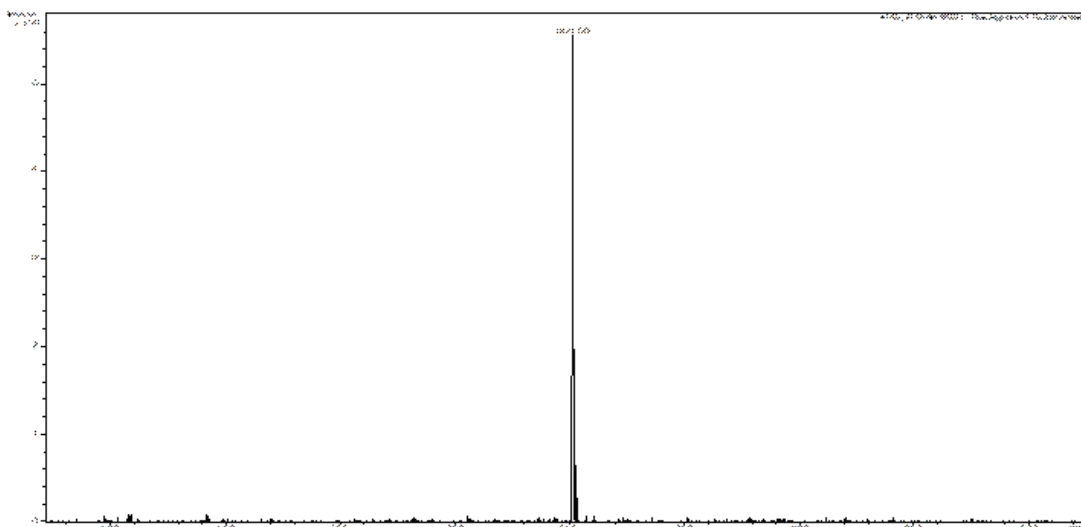


Figure 3: Mass spectra of N-(2-(methylamino) ethyl)-2-methoxy-5-(methyl sulfonyl) benzamide: Observed mass 301 at retention time of 5.44 min

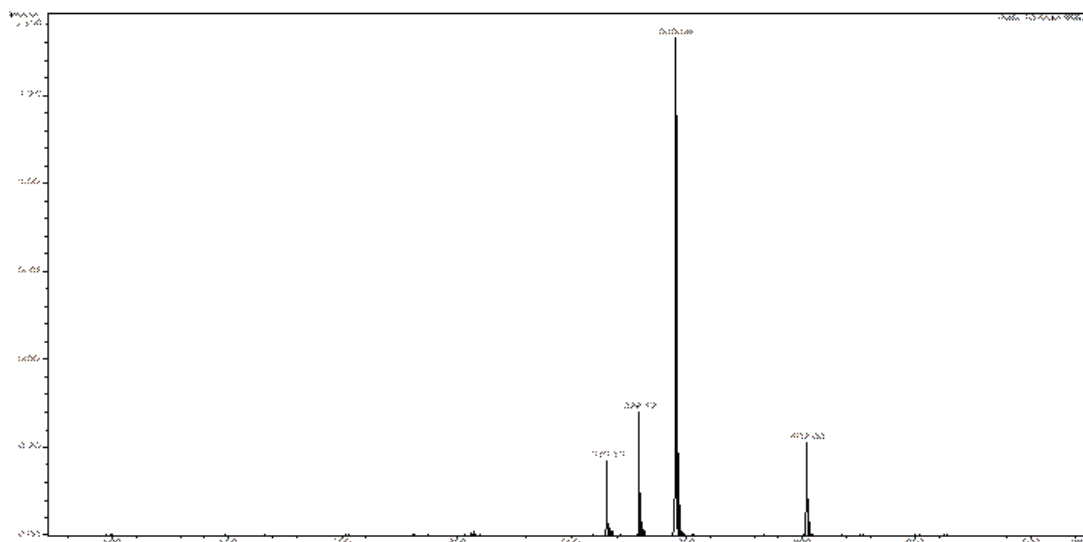


Figure 4: Mass spectra of Tiapride N- oxide: Observed mass 345 at retention time of 9.95 min

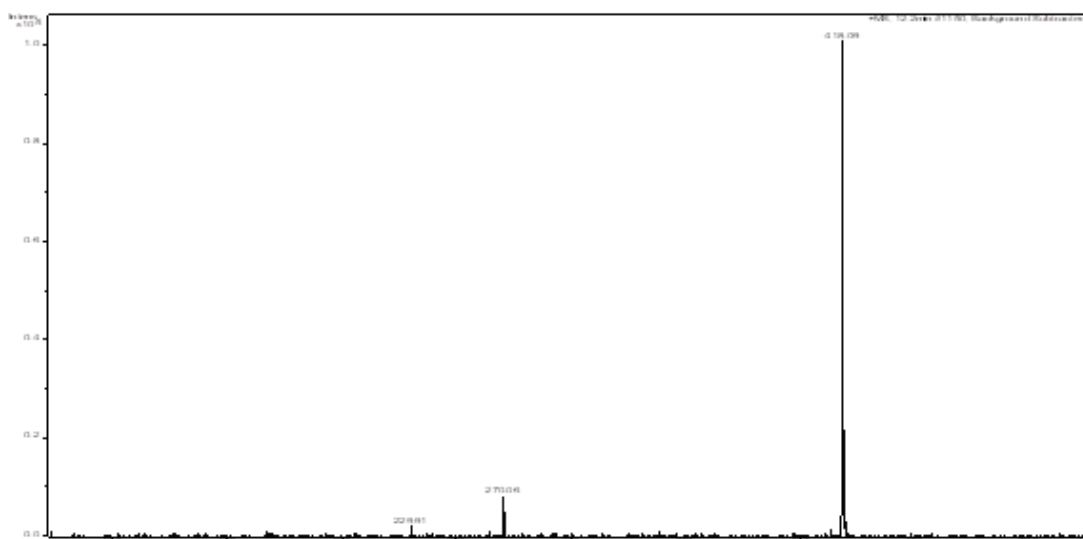


Figure 5: Mass spectra of 2-Methoxy-5-sulfonyl benzoic acid 229 mass at retention time of 12.48 min

LC-MS analysis confirmed the presence of three distinct impurities (Figure 3, 4, 5), each well resolved and exhibiting m/z values consistent with their respective molecular weights, as summarized in

(Table 3) These degradation products were observed at different retention times and were successfully identified based on their accurate mass measurements.

Table 3: Degradation impurities m/z fragmentation

Impurities	Molecular /fragment m/z	Molecular formula	Positive ion	Molecular weight	Retention time (min)
Tiapride N-oxide	[M+H] ⁺	C ₁₅ H ₂₄ N ₂ O ₅ S	345	344	9.95
N-(2-(ethylamino)ethyl)-2-methoxy-5-(methylsulfonyl)benzamide	[M+H] ⁺	C ₁₅ H ₂₀ N ₂ O ₄ S	301	300	5.44
2-Methoxy-5-sulfonyl benzoic acid	[M+H]	C ₉ H ₁₀ O ₅ S	230	229	12.48

To further validate these findings, the impurities were isolated using preparative chromatography. The collected fractions were then subjected to detailed structural elucidation using nuclear magnetic resonance (NMR) spectroscopy, confirming the identity of each degradation product.

¹H and ¹³C NMR Characterization: Tiapride Hydrochloride

The ¹H and ¹³C NMR spectra of Tiapride Hydrochloride confirmed the presence of key aromatic and aliphatic structural elements that align with its known molecular structure. The ¹H NMR spectrum showed 23 proton signals, while the ¹³C NMR revealed 11 carbon signals, matching the expected atom count of the compound. In the aromatic region, three distinct multiplets were observed: δ

7.99–8.00 ppm (H1, C2), δ 7.37–7.39 ppm (H1, C3), and δ 8.22–8.23 ppm (H1, C6), indicating a substituted benzene ring. These were linked to CH carbons in the ¹³C spectrum at δ 132.0, 113.5, and 123.6 ppm, respectively. The amide NH proton appeared as a doublet at δ 7.99–8.02 ppm (H1, C8), suggesting coupling with nearby protons. In the aliphatic region, a distinct triplet of methylene and methyl signals was noted. A methylene doublet at δ 3.84–3.96 ppm (H3, C11) was attributed to the N-CH₂ group, with a corresponding ¹³C signal at δ 57.2 ppm (CH₂).

Additional methylene groups were found between δ 2.49–3.96 ppm, with ¹³C resonances at δ 50.0, 47.3, 44.3, and 39.7 ppm (C15–C21), consistent with a flexible side chain. The terminal methyl groups (C20, C22) showed singlets at δ 1.152 ppm in ¹H and δ 10.7 ppm in ¹³C NMR,

confirming their isolated environment. A significant amide carbonyl carbon signal was detected at δ 164.6 ppm (C7), indicative of the CONH, group, while another carbonyl-type signal at δ 161.0 ppm (C4) suggested an electron-withdrawing substituent, likely a sulfonyl or amide linkage. Additional quaternary carbons appeared at δ 133.0, 130.2, and

164.6 ppm, further supporting the substituted aromatic ring and heteroatom linkage. Overall, the NMR data verified the intact structure of tiapride hydrochloride, with a combination of aromatic protons, amide functionality, and an aliphatic side chain featuring methylene and terminal methyl groups (Table 4).

Table 4: ^1H and ^{13}C Chemical Shift of Tiapride Hydrochloride

Atom Position	^1H Multiplicity (δ ppm)	^{13}C (δ ppm)	DEPT
1	-	133.0	-
2	7.99-8.0 (m, H1)	132.0	CH
3	7.37-7.39 (m, H1)	113.5	CH
4	-	161.0	-
5	-	130.2	-
6	8.22-8.23 (m, H1)	123.6	CH
7	-	164.6	-
8	7.99-8.02 (d, NH)	-	-
9	-	-	-
10	-	-	-
11	3.841-3.96 (dd, H3)	57.2	CH ₃
12	-	-	
13	-	-	
14	-	-	

15	3.0-3.16 (d, H2)	47.3	CH ₂
16	3.84-3.96 (d, H2)	39.7	CH ₂
17	2.493-2.50 (d, H2)	50.0	CH ₂
18	-	-	
19	2.493-2.50 (d, H2)	44.3	CH ₂
20	1.152 (s, H3)	10.7	CH
21	2.497-2.50 (d, H2)	44.3	CH ₂
22	1.152 (s, H3)	10.7	CH ₃

Structural Elucidation of Degradation Impurity

DP1: Mass spectrometry

The mass spectrum of tiapride hydrochloride exhibited a prominent peak at m/z 329.1 in positive ion mode, corresponding to the $[M+H]^+$ ion. This indicated a molecular weight of 328 g/mol for the parent compound. In comparison, the mass spectrum of DP1 showed a protonated molecular ion peak at m/z 301 ($[M+H]^+$), indicating a molecular weight of 300 g/mol—28 Da lower than that of tiapride. This mass difference suggested the loss of an ethyl group or a portion of the aliphatic side chain during degradation (Table 3) (Figure 3).

DP1: Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^1H and ^{13}C NMR spectra of tiapride confirmed the presence of 23 protons and

11 carbon atoms, respectively. In contrast, DP1 displayed signals corresponding to 20 protons and 9 carbon atoms, indicating structural truncation during degradation.

In the ^{13}C NMR spectrum of tiapride, a peak at δ 164.6 ppm (C7) was assigned to the amide carbonyl (CONH_2) group. Additional peaks at δ 44.3 ppm (C19, C21) corresponded to the aliphatic side-chain methylene carbons, while the δ 132.0 ppm signal (C2) represented an aromatic carbon.

In the ^{13}C NMR spectrum of DP1, a single signal at δ 44.3 ppm was observed for an aliphatic carbon (C19), along with the δ 164.6 ppm peak attributed to the amide carbonyl group (C7). The absence of one of the aliphatic signals suggested that one side chain was cleaved during degradation, supporting the observed mass loss.

The ^1H NMR spectrum of tiapride showed a signal at δ 8.02 ppm, corresponding to

the amide proton (H8), confirming the presence of the CONH₂ group. In DP1, the ¹H NMR spectrum displayed a signal at δ 7.38 ppm (H12) attributed to the amide proton and a second signal at δ 2.01 ppm

(H18), corresponding to a secondary amine (NH) proton.

These spectral differences clearly indicate structural modification of the aliphatic chain in DP1 (Table 5).

Table 5: ¹H and ¹³C Chemical Shift of DP1 (N-(2-(ethyl amino) ethyl)-2-methoxy-5-(methyl sulfonyl) benzamide)

Atom Position	¹ H Multiplicity (δ ppm)	¹³ C (δ ppm)	DEPT
1	-	133.0	-
2	7.38 - 8.0 (m, H1)	132.0	CH
3	7.38 - 7.41 (d, H1)	113.5	CH
4	-	161.0	
5	-	130.2	
6	8.25 (d, H1)	123.6	CH
7	-	-	
8	-	-	
9	-	-	
10	3.43 - 3.53 (d, H3)	57.2	CH ₃
11	-	164.6	-
12	7.38 - 8.0 (d, NH)	-	-
13	-	-	-
14	-	-	-
15	3.98 (s, H3)	57.2	CH ₃
16	3.43 - 3.537 (d, H2)	40.2	CH ₂
17	2.49 (d, H2)	47.3	CH ₂
18	2.06 - 2.07 (d, NH)	-	-
19	2.49 (d, H2)	44.3	CH ₂
20	1.02 - 1.22 (d, H3)	8.7	CH ₃

Whereby, s: singlet; d: doublet; m: multiplet; ss: singlet of singlet; dd: doublet of doublet; dm: double multiplet.

DP1: Structure Confirmation

Based on the LC-MS and NMR analysis, the molecular formula of DP1 was confirmed as $C_{13}H_{20}N_2O_4S$, and its chemical structure was identified as: N-(2-(ethylamino)ethyl)-2-methoxy-5-(methylsulfonyl)benzamide (Figure 6).

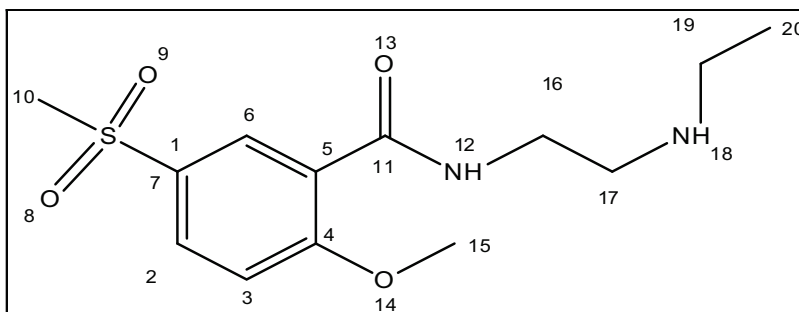


Figure 6: N-(2-(ethyl amino) ethyl)-2-methoxy-5-(methyl sulfonyl) benzamide (DP1)

DP2: Mass spectrometry

The ESI mass spectrum of DP2 displayed a deprotonated molecular ion $[M-H]^-$ at m/z 229, indicating a molecular weight of 230 g/mol, which is 99 Da lower than that of tiapride (Table 3). A minor fragment ion was also detected at m/z 229, consistent with the proposed molecular structure (Figure 5).

DP2: Nuclear Magnetic Resonance (NMR) Spectroscopy

The ^{13}C NMR spectrum of DP2 showed the absence of signals corresponding to the amide group and aliphatic chain. Notably, the amide carbonyl signal at δ 164 ppm (C7), as well as the aliphatic carbon signals at δ 37.5 ppm (C16) and δ 50.0 ppm (C17),

were no longer present. In addition, the characteristic methyl carbon signals at δ 10.7 ppm (C20 and C22)—attributable to terminal ethyl groups in tiapride—were also absent. These spectral changes confirmed the loss of both the amide moiety and the diethylaminoethyl side chain in DP2.

The ^1H NMR spectrum of DP2 exhibited signals corresponding to 10 protons. Aromatic proton signals were observed at δ 7.99 ppm (H2), δ 7.37 ppm (H3), and δ 8.22 ppm (H6), assigned to the benzene ring. The chemical shifts for other protons in the 2-methoxy-5-(methylsulfonyl) ring closely resembled those of the parent compound, supporting retention of the core aromatic scaffold (Table 6).

Table 6: ¹H1 and ¹³C Chemical Shift of DP2 (2-Methoxy-5-sulfonyl benzoic acid)

Atom Position	¹ H Multiplicity (δ ppm)	¹³ C (δ ppm)	DEPT
1	-	132.0	-
2	7.99 - 8.01 (d, H1)	133.02	CH
3	7.07 - 7.48 (dm, H1)	113.5	CH
4	-	161.0	-
5	-	123.6	-
6	8.22 - 8.40 (dm, H1)	130.2	CH
7	-	-	-
8	-	-	-
9	-	-	-
10	3.30 - 3.35 (d, H3)	47.3	CH ₃
11	-	171.8	-
12	-	-	-
13	-	-	-
14	-	-	-
15	3.80-3.97 (d, H3)	57.2	CH ₃

Whereby, d: doublet; dm: double multiplet.

DP2: Structure Confirmation

Based on the LC-MS and NMR analysis, the chemical structure of DP2 was

confirmed as: 2-methoxy-5-sulfonylbenzoic acid, with the molecular formula C₉H₁₀O₅S (Figure 7).

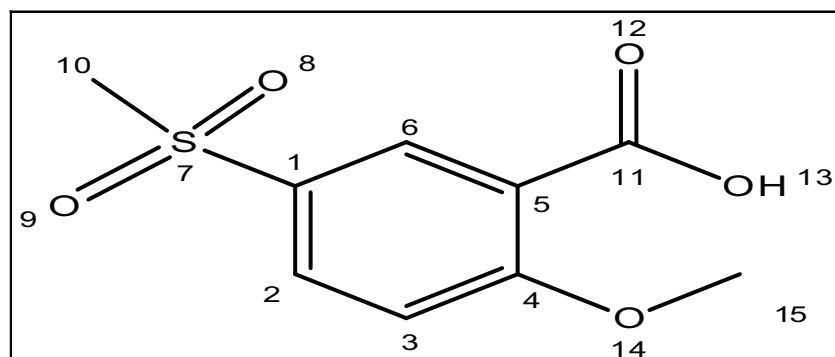


Figure 7: 2-Methoxy-5-sulfonyl benzoic acid (DP2)

DP3: Mass spectrometry

The ESI mass spectrum of DP3 exhibited a protonated molecular ion $[M+H]^+$ at m/z 345, indicating a molecular weight of 344 g/mol—16 Da higher than that of the parent compound tiapride. This mass difference suggested the incorporation of one oxygen atom, likely due to oxidation at the nitrogen atom of the diethylamino side chain (Table 3).

Two fragment ions were also observed: one at m/z 329, indicating the loss of an oxygen atom, and another at m/z 315, corresponding to the loss of an oxygen atom and a methyl group (CH_3) (Figure 4).

DP3: Nuclear Magnetic Resonance (NMR) Spectroscopy

In the ^{13}C NMR spectrum of DP3, a signal for the amide carbonyl group appeared at δ 164.2 ppm (C7). Aliphatic carbon signals were detected at δ 40.3 ppm (C12, C14), while the aromatic carbon (C2) resonated at δ 133 ppm, similar to the parent compound.

The ^1H NMR spectrum of DP3 showed signals corresponding to 24 protons. A peak at δ 7.67 ppm (H8) was attributed to the amide proton ($CONH_2$). Additional aromatic proton signals appeared at δ 6.54 ppm (H2), δ 7.47 ppm (H3), and δ 6.94 ppm (H6), assigned to protons on the benzene ring. The chemical shifts observed for the 2-methoxy-5-(methylsulfonyl) moiety closely matched those of tiapride, indicating that the core structure remained intact (Table 7).

Table 7: ¹H and ¹³C Chemical Shift of DP3 (N-[2-(Diethylnitro)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide) (Tiapride N Oxide)

Atom Position	¹ H Multiplicity (δ ppm)	¹³ C (δ ppm)	DEPT
1	-	130.2	-
2	6.54 (s, H1)	133.0	CH
3	7.47 - 7.67 (d, H1)	113.5	CH
4	-	161.0	-
5	-	123.6	-
6	6.94 - 7.20 (dm, H1)	132.0	CH
7	-	164.6	-
8	7.67 - 7.70 (d, NH)	-	-
9	4.11 (dm, H2)	-	CH ₂
10	2.49 - 2.50 (d, H2)	57.2	CH ₂
11	-	-	-
12	2.49 - 2.50 (d, H2)	40.3	CH ₂
13	0.82 - 1.64 (dm, H3)	8.9	CH ₃
14	2.49 - 2.50 (d, H2)	40.3	CH ₂
15	0.82 - 1.64 (dm, H3)	8.9	CH ₃
16	-	-	
17	-	-	
18	-	-	
19	-	-	
20	-	-	
21	3.34 (d, H3)	47.3	CH ₃
22	-	-	
23	3.99 - 4.19 (m, H3)	50.0	CH ₃

Whereby, s: singlet; d: doublet; m: multiplet; ss: singlet of singlet; dd: doublet of doublet; dm: double multiplet.

DP3 Structure Confirmation

Based on the LC-MS and NMR analysis, DP3 was identified as: N-[2-

(diethylnitroso)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide (Tiapride N-oxide) with the molecular formula $C_{15}H_{24}N_2O_5S$ (Figure 8).

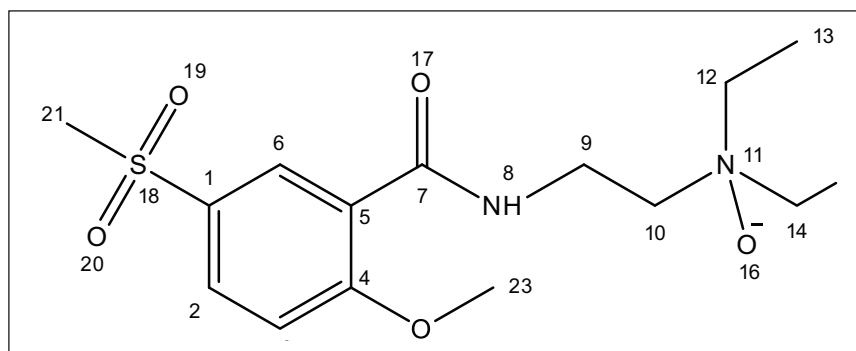


Figure 8: N-[2-(Diethylnitroso)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide (Tiapride N Oxide) (DP3)

Table 8 summarizes the key physicochemical characteristics of tiapride and its three degradation products (DP1, DP2, DP3) as elucidated through LC/MS and NMR analyses. The table presents comparative data on molecular formulas, ion masses, and diagnostic ^1H and ^{13}C NMR chemical shifts, highlighting the

structural modifications observed in each degradation product relative to the parent compound. This comparative summary serves as a consolidated reference, confirming the structural identity of each impurity and supporting the proposed degradation pathways under various stress conditions.

Table 8: Summary of LC/MS and NMR Data for Tiapride and Its Degradation Products

Compound	Tiapride	DP1	DP2	DP3
Molecular Formula	C ₁₅ H ₂₄ N ₂ O ₄ S	C ₁₃ H ₂₀ N ₂ O ₄ S	C ₉ H ₁₀ O ₅ S	C ₁₅ H ₂₄ N ₂ O ₅ S
[M+H] ⁺ / [M-H] ⁻ (m/z)	329.1 ([M+H] ⁺)	301 ([M+H] ⁺)	229 ([M-H] ⁻)	345 ([M+H] ⁺)
Molecular Weight (Da)	328	300	230	344
Key ¹ H NMR Signals (δ, ppm)	8.02 (H8, amide), aromatic at ~7.3–7.9	7.38 (CONH ₂), 2.01 (NH)	8.22, 7.99, 7.37 (aromatic)	7.67 (H8, amide), 6.54, 7.47, 6.94 (aromatic)
Key ¹³ C NMR Signals (δ, ppm)	164.6 (C=O), 44.3 (aliphatic), 132 (aromatic)	164.6 (C=O), 44.3 (aliphatic)	No C=O (amide), no aliphatic CH ₂ , CH ₃ signals	164.2 (C=O), 40.3 (aliphatic), 133 (aromatic)
Structural Features	Intact structure with diethylaminoethyl side chain	Loss of one ethyl group; side chain cleavage	Amide and side chain hydrolyzed; benzoic acid formed	Oxidation at nitrogen; N→O bond formation (N-oxide)

Degradation Behavior and Proposed Mechanisms of Tiapride Hydrochloride

Tiapride hydrochloride exhibits chemical stability under standard storage conditions; however, it demonstrates susceptibility to degradation under forced stress environments. Significant degradation is observed under acidic, basic, and oxidative conditions, each promoting distinct transformation pathways.

Hydrolytic Degradation (Acidic and Basic Conditions): Under both acidic and basic

hydrolytic conditions, the amide bond in the parent compound undergoes cleavage, resulting in the formation of 2-methoxy-5-methylsulfonylbenzoic acid (Figure 7). This reaction is attributed to the nucleophilic attack of water on the carbonyl carbon of the amide, leading to bond scission and formation of the corresponding carboxylic acid.

In addition, the methoxy group, located ortho to the amide and para to the sulfonyl moiety, exhibits vinylogous ester behavior. This makes it susceptible to hydrolytic

cleavage, resulting in the generation of N-(2-diethylaminoethyl)-2-hydroxy-5-methylsulfonylbenzamide (Figure 8). This transformation is likely facilitated by the activation of the aryl ether system under aqueous stress conditions.

Oxidative Degradation: Upon exposure to oxidative stress—particularly in the presence of hydrogen peroxide (H₂O₂)—the molecule undergoes site-specific oxidation at the tertiary amine functional group within the diethylaminoethyl side chain. This results in the formation of an N-oxide derivative, identified as N-(2-diethylaminoethyl)-2-methoxy-5-methylsulfonylbenzamide N-oxide (Figure 6).

The proposed mechanism involves nucleophilic attack by the lone pair of electrons on the nitrogen atom of the diethylamino group upon the electrophilic oxygen of the oxidizing agent. The process progresses through a transient intermediate, followed by proton transfer and subsequent stabilization, ultimately forming an N→O bond characteristic of N-oxide functionality.

CONCLUSION

The present study demonstrates the successful application of advanced analytical methodologies—namely high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy—for the detection, isolation,

and structural elucidation of unknown degradation impurities associated with tiapride hydrochloride. A stability-indicating HPLC method enabled the identification and resolution of three previously unreported degradation products (DP1, DP2, and DP3), with DP3 being predominantly formed under oxidative stress conditions involving hydrogen peroxide. These impurities were generated under forced degradation conditions, purified using preparative HPLC, and characterized using LC-MS and comprehensive NMR analysis (¹H and ¹³C). The identified structures were as follows: DP1 was characterized as *N*-(2-(ethylamino)ethyl)-2-methoxy-5-(methylsulfonyl)benzamide, DP2 as 2-methoxy-5-sulfonylbenzoic acid, and DP3 as *N*-[2-(diethylnitroso)ethyl]-2-methoxy-5-(methylsulfonyl)benzamide, also referred to as tiapride n-oxide. To the best of our knowledge, this is the first report describing the isolation and structural confirmation of these specific degradation products. Furthermore, accelerated stability studies under simulated long-term storage conditions provided essential insights into the degradation behavior of Tiapride, thereby contributing to the establishment of suitable storage guidelines. Collectively, this study enhances the understanding of Tiapride's stability profile and offers valuable data to support quality assurance in pharmaceutical development and regulatory compliance.

ABBREVIATIONS

Å: Angstroms

AR: Analytical Reagent

API: Active Pharmaceutical Ingredients

°C: Degree Celsius

Da: Dalton

DEPT: Distortionless Enhancement by Polarization Transfer

δ: Delta

H₂O₂: Hydrogen Peroxide

HCl: Hydrochloric Acid

HILIC: Hydrophilic Interaction Liquid Chromatography

hr: Hour

ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

LC: Liquid Chromatography

LC/MS and LCMS: Liquid Chromatography Mass Spectrometry

MHz: Mega Hertz

mL: Milliliter

m/z: Mass-to-Charge Ratio

Min: Minute

M: Molar

mm: Millimeter

µg: Micro gram

µL: Microliter

µm: Micrometer

MS: Mass Spectrometry

NaOH: Sodium Hydroxide

ND: Not Detected

nm: Nano meter

NMR: Nuclear Magnetic Resonance

ppm: Parts Per Million

RH: Relative Humidity

RP-HPLC: Reverse Phase High Performance Liquid Chromatography

RRT: Relative Retention Time

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