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Original Article

Forced degradation behavior of epidepride and development of a stability-indicating method based on liquid chromatography–mass spectrometry

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ABSTRACT

A reversed-phase high-performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was applied to study the forced degradation behavior and stability of epidepride. ^{123}I radioisotope-labeled epidepride, [(-)-N-{[(2S)-1-ethylpyrrolidin-2-yl]methyl}-5-iodo-2,3- dimethoxybenzamide] is a radiotracer with a high affinity for dopamine D_2 receptors in the brain and has been used as an imaging agent for single-photon emission computed tomography. HPLC studies were performed using ^{127}I-epidepride (the nonradioactive compound), instead of ^{123}I-epidepride, with an RP-18 column using a mobile phase consisting of methanol, acetonitrile, and ammonium acetate (pH 7.0, 10 mM). The eluent flow rate and the wavelength for HPLC detection were 0.5 mL/min and 210 nm, respectively. The ligand was exposed to acid (1 N HCl) and alkaline (1 N NaOH) media and was subjected to oxidative decomposition at room temperature using 3% H_2O_2 and to thermal decomposition at 50°C. After various reaction times (30 minutes, 1 hour, 2 hours, 8 hours, and 24 hours), the substances were investigated by HPLC and LC-MS/MS. Although no decomposition products were observed after the acidic, alkaline, and thermal treatments, >80% of the initial amount of ^{127}I-epidepride was oxidized within 24 hours in the presence of H_2O_2. Only one major oxidation product with an m/z value of 435 was observed, in addition to the ^{127}I-epidepride species (m/z 419). The product was characterized by LC-MS/MS fragmentation, and the deteriorated type and fragmentation pathways were proposed for epidepride.

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1. Introduction

Dopamine belongs to a family of neurotransmitters, along with serotonin, acetylcholine, and norepinephrine, which is released from the central nervous system (CNS), which includes the midbrain, substantia, and hypothalamus [1]. In mammals, dopamine plays an important role in controlling movement, memory, pleasure, reward, sleep, mood, learning, behavior, and cognition, among others [2]. An excess or a deficiency of this vital chemical may lead to various disorders including Parkinson's disease and drug addiction [3−5]. Effects of dopamine occur via dopamine receptors, which transmit neural signals and regulate many activities. Therefore, dopamine receptors are the targets of common neurological drugs. There are five subtypes of dopamine receptors that are present at higher levels: D_1, D_2, D_3, D_4, and D_5 [6], but D_1 and D_2 are the major subtypes, accounting for >90% of all the dopamine receptors. The D_2 dopamine receptor serves as an information transmitter and neurotransmission regulator; hence, the development of a new D_2 receptor antagonist drug for the treatment and/or diagnosis of CNS disorders is important.

Epidepride, (S)-(-)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (Fig. 1), is one of the members of a family of compounds with a high affinity for the dopamine D_2 receptor antagonist [7]. Radioisotopes of iodine ($^{123/125}$I), which emit γ radiation, have been used to substituted ^{127}I in epidepride, leading to $^{123/125}$I-epidepride; a compound that was first developed by Kessler et al [8] in 1991, to study precisely the activity and distribution of the D_2 receptor in the brain during Parkinson's disease, by single photon emission computed tomography (SPECT) [9]. Epidepride was originally derived from the structures of (S)-sulpiride and remoxipride. ^{123}I-epidepride is better than ^{123}iodobenzamide, which is another D_2-receptor imaging-agent candidate with high specificity and affinity [10,11]. de Herder et al [10] have reported that ^{123}I-epidepride scans allow a consistently superior visualization of pituitary adenoma, and suggested that this compound could potentially replace ^{123}I-iodobenzamide in future studies of D_2-receptors by SPECT. Leslie et al [11] and Huang et al [5] also showed that ^{123}I-epidepride is a promising radiotracer for evaluating the therapeutic effects of schizophrenia. Pirker et al [12] reported a high sensitivity (in the picomole range) of ^{123}I-epidepride-based SPECT, which can be applied to predict the effects of dopamine treatment on pituitary tumors.
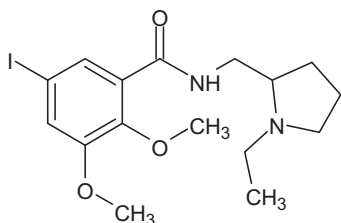
According to the current International Conference on Harmonization (ICH) "Stability Testing Guidelines", a new drug should undergo a series of analytical tests, and the assays should be fully validated. Moreover, the guidelines suggest that the drug should be subjected to stress testing or forced degradation studies [13]. Stress testing of a drug is used to survey the stability of a given system or an entity. It involves testing the substance beyond normal operational conditions. By contrast, a stability indicating method is defined as a part of validated analytical methods that can accurately and precisely quantitate the decrease in active pharmaceutical ingredients due to degradation [14]. Therefore, it is important to be able to identify the possible degradation products, which is in turn helpful for determining the degradation pathways and the intrinsic stability of the molecule and for validating the stability − indicating the power of the drug during analytical procedures. According to the guidelines, the test should also include the effects of temperature, humidity (where appropriate), oxidation, photolysis, and susceptibility to hydrolysis across a wide pH range. Stability testing aims to evaluate the quality of a drug, determine whether the product changes with time under various environmental factors, and establish a retesting period for the substance or recommend a shelf life and appropriate storage conditions for the drug [15]. Information about the degradation pathways and the degradation products that can possibly form during storage or manufacturing processes may improve pharmaceutical safety, in which a better control of the chemical behavior of a drug could help maintain its quality [16].

This study established high-performance liquid chromatography (HPLC) and liquid chromatography−tandem mass spectrometry (LC-MS/MS) methods to monitor the rate and identify the structure of the degradation products formed during stress-testing studies of epidepride. We studied the inherent stability of the compound through stress studies carried out under different conditions, including thermal, acidic/alkaline hydrolysis, and oxidative to determine the stability and ensure the quality of this chemical compound, which serves as an active pharmaceutical ingredient. To follow the regulatory requirements for radiation safety programs ("As Low As Reasonably Achievable", ALARA), ^{127}I-epidepride was used instead of ^{123}I or ^{125}I-labeled epidepride in the study.

2. Materials and methods

2.1. Materials and reagents

Analytical-grade laboratory chemicals for HPLC were used as received without further purification. Methanol, acetonitrile (both HPLC grade), ammonium acetate, sodium hydroxide, hydrochloric acid and H_2O_2 (30%) were all purchased from Merck (Darmstadt, Germany). Deionized water was purified using a Smart DQ3 reverse osmosis reagent water system (Merck Millipore, Billerica, MA, USA) with a 0.22-μm filter, total organic carbon (TOC) <5 ppb, resistivity ≥18 MΩ cm, and endotoxin ≤0.001 EU/mL. ^{127}I-epidepride was synthesized and purified, following the procedures briefly described in the next

Fig. 1 − Structure of ^{127}I-epidepride, $C_{16}H_{23}N_2O_3I$ − (molecular weight = 418.3).
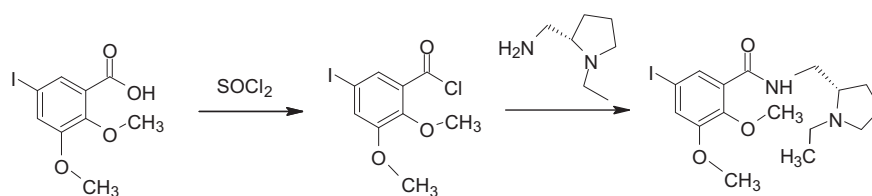
Fig. 2 – Scheme for the preparation of epidepride.

section, by the Chemistry Division, Institute of Nuclear Energy Research (INER; Longtan Township, Taoyuan County, Taiwan, R.O.C.). Reagent-grade laboratory chemicals for the synthesis were obtained from commercial sources. 5-Iodo-2,3-dimethoxy benzoic acid, thionyl chloride, bis(triphenyl-phosphine)-palladium(II) chloride, and (S)-(-)-2-aminomethyl-1-ethylpyrrolidine were purchased from Merck. Infrared spectra were obtained on a Bio-Rad FTS-40 spectrometer (Bio-Rad Co., Hercules, CA, USA). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 2000 spectrometer (300 MHz) (Varian Inc., Palo Alto, CA, USA). Gas chromatography was performed on a Shimadzu GCMS-QP 1100EX (Shimadzu Co., Kyoto, Japan).

2.2. Synthesis procedures and spectra of ^{127}I-epidepride

A solution of 5-iodo-2,3-dimethoxybenzoic acid (1.0 g, 3.2 mmol) in thionyl chloride (10 mL) was heated under reflux for 2 hours, and concentrated under reduced pressure. The residue was redissolved in dichloromethane (20 mL), and the solution was cooled to around 4°C. (S)-(-)-2-Aminomethyl-1-ethylpyrrolidine (0.41 g, 3.2 mmol) in dichloromethane (10 mL) was added dropwise, and the resultant solution was stirred for 3 hours at room temperature, washed thoroughly with 1 N NaOH (20 mL), dried over anhydrous Na$_2$SO$_4$, concentrated, and finally purified by chromatography (SiO$_2$, dichloromethane/methane = 19:1) to yield epidepride (1.27 g, 95%). The synthesis reaction scheme is shown in Fig. 2 [17,18].

Spectra of epidepride: IR (neat) ν 3376 (NH), 1659 (C=O) cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 8.32 (br, 1 H, NH), 8.03 (d, J = 2.1 Hz, 1 H, Ph-H), 7.29 (d, J = 2.1 Hz, 1 H, Ph-H), 3.89 (s, 3 H, OCH$_3$), 3.88 (s, 3 H, OCH$_3$), 3.76 (ddd, J = 13.8, 7.2, and 2.4 Hz, 1 H, NHCH$_2$), 3.30 (ddd, J = 13.8, 5.1, and 2.7 Hz, 1 H, NHCH$_2$), 3.19 (m, 1 H, NCH$_2$CH$_2$), 2.86 (m, 1 H, NCH$_2$CH$_3$), 2.64 (m, 1 H, NCH), 2.21 (m, 2 H, CH$_2$CH$_3$ and NCH$_2$CH$_2$), 1.88 (m, 1 H, CHCH$_2$), 1.69 (m, 3H, NCH$_2$CH$_2$CH$_2$), 1.12 (t, J = 7.2 Hz, 3 H, CH$_2$CH$_3$); ^{13}C NMR (CDCl$_3$) δ 163.76 (CO), 153.15, 147.56, 131.64, 128.27, 123.85, and 87.09 (Ph), 62.11 (CH), 61.19 (OCH$_3$), 56.24 (OCH$_3$), 53.35, 47.71, 40.92, 28.20, and 22.50 (CH$_2$), 13.86(CH$_3$); GC-MS m/z 418 (M$^+$), 403 (M$^+$-CH$_3$), 389 (M$^+$-CH$_2$CH$_3$), 320 (M$^+$-CH(CH$_2$)$_3$NCH$_2$ CH$_3$), 291 (M$^+$-NHCH$_2$CH(CH$_2$)$_3$NCH$_2$CH$_3$).

2.3. Apparatus and equipment

2.3.1. HPLC instrumentation
Quantification and studies of the degradation behavior of epidepride were performed on an Agilent 1100 series HPLC system (Agilent, Palo Alto, CA, USA), which consisted of an on-line degasser, a binary pump, an autosampler, a thermostated column oven, and a photodiode array detector (PDA). The data

were acquired and processed using Agilent ChemStation software (Agilent), and a porous monolithic silica (with bimodal pore structure, i.e., macropores with 2 μm and mesopores 13 nm in diameter) surface modified with octadecylsilan and full end-caping reversed-phase column, according to the manufacturer instructions (Chromolith Performance RP-18e, 4.6 × 100 mm, Merck, Darmstadt, Germany), was used for LC separation. The column temperature was maintained at 25°C. Isocratic elution was achieved using a mobile phase consisting of methanol and acetonitrile (15:85, v/v) added with ammonium acetate (pH 7.0, 10 mM) as buffer. The flow rate was 0.5 mL/min, and the injection volume was 5 μL. The optimal wavelength for the detection and quantification of the drug and its degradation products was set at 210 nm.

2.3.2. LC-MS/MS instrumentation
Mass spectrometric analysis was carried out on a 4000 QTrap LC-MS/MS system, containing the API Analyst software 1.4.1 (MDS Sciex, Vaughan, Ontario, Canada), to study the fragmentation ions and the forced degradation product of epidepride. The samples were introduced into the spectrometer either by the HPLC system (Agilent 1100) or by a syringe pump at a flow rate of 10 μL/min (Harvard, Harvard Apparatus, Holliston, MA, USA). Chromatographic separation was conducted as described in the section on HPLC conditions. The analytes were ionized by a turbo spray ion source (electrospray ionization) in the positive-ion mode at 5500 V and 500°C. Mass spectra were recorded within the range of 50–1000 amu with unit resolutions in Q1 and Q3. Other experimental parameters are outlined in Table 1. Determination of the epidepride concentration by LC-MS was conducted in the

Parameters	Epidepride
Source temperature (°C)	500
Polarity	Positive
Resolution, Q1 and Q3	Unit
Nebulizer gas, NEB (psi)	45
Curtain gas, CUR (psi)	10
Turbo gas	50
Collision gas, CAD (psi)	Medium
Ion spray voltage, IS (V)	5500
Ion energy 1, IE1 (V)	0.6
Ion energy 3, IE3 (V)	0.2
Declustering potential, DP (V)	100
Entrance potential, EP (V)	10
Detector parameter	Positive
- Channel electron multiplier, CEM (V)	1950

Table 1 – Tandem mass spectrometry parameters for epidepride determination.
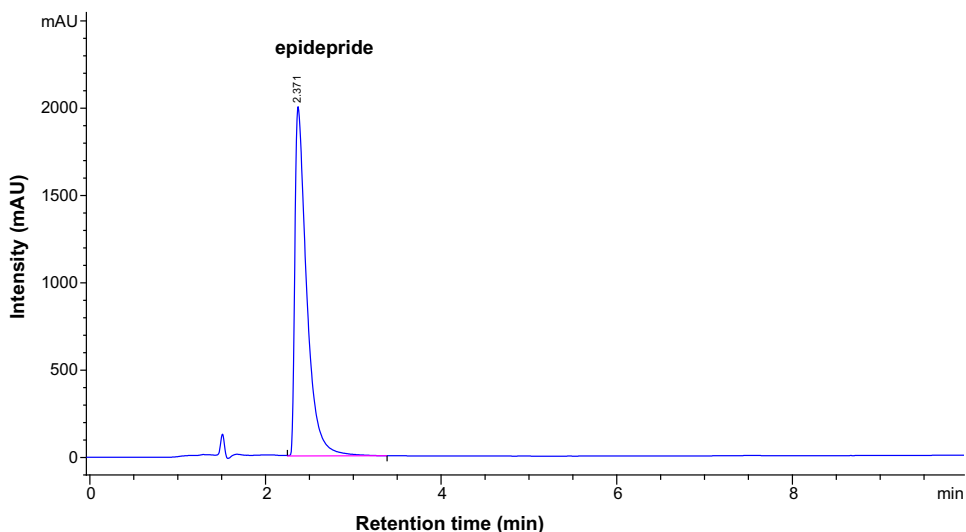
Fig. 3 − High-performance liquid chromatography of epidepride.

multiple reaction monitoring (MRM) positive-ion mode. Nitrogen was used as the nebulization, curtain, and collision gas in all the 4000 QTrap LC−MS/MS studies.

2.3.3. Other equipment

A pH meter (Sartorius AG, Goettingen, Germany) was used to check and adjust the pH of the buffer solutions. Temperature accuracy and constancy of the oven (Binder GmbH, Tuttlingen, Germany) were previously calibrated and then used for the subsequent thermal-stress study.

2.4. Procedures

To verify that the HPLC analytical method was suitable to determine epidepride, including linearity, precision, and accuracy were determined according to the guidelines: "ICH Topic Q 2 (R1): Validation of analytical procedures: text and methodology" issued by the European Medicines Agency [16].

2.4.1. Linearity

To establish analytical calibration linearity and the appropriate range for the epidepride studies by HPLC, a stock solution containing 1000 μg/mL drug in methanol was prepared. This stock solution was then diluted to yield analyte solutions

in the concentration range of 1−100 μg/mL, which were freshly prepared daily. The solutions were injected in triplicate into the HPLC system, and the calibration curve was then obtained by plotting the respective peak areas against the concentrations. Quality control samples of 10 μg/mL, 50 μg/mL, and 75 μg/mL were similarly prepared. Linearity was evaluated by the linear least-square regression method. The calibration curves for epidepride through MRM positive-ion detection mode using LC-MS/MS were also established between the concentration range of 1−100 ng/mL.

2.4.2. Precision

Intra-day precision was established by analyzing 10 μg/mL, 50 μg/mL, and 75 μg/mL epidepride solutions in triplicate on the same day and calculating the values of the relative standard deviation (RSD). The studies were also repeated on different days to determine inter-day precision.

2.4.3. Accuracy

The accuracy was assayed by the recovery test. Samples were spiked with three known amounts of epidepride (10 μg/mL, 50 μg/mL, and 75 μg/mL) in triplicate, and then the percentage recovery of the added drug was determined.

2.4.4. Stress studies

Stress studies of epidepride were carried out under different conditions including acidic and alkaline hydrolysis,

Table 2 − Data of intra-day and inter-day precision studies ($n = 3$).		
Concentration (μg/mL)	Intra-day measured concentration (μg/mL) ± SD; RSD% ($n = 3$)	Inter-day measured concentration (μg/mL) ± SD; RSD% ($n = 3$)
10	10.450 ± 0.124; 1.182	10.662 ± 0.212; 1.990
50	49.078 ± 0.158; 0.322	48.541 ± 0.145; 0.298
75	77.909 ± 1.325; 1.701	77.956 ± 1.391; 1.784
RSD = relative standard deviation; SD = standard deviation.		

Table 3 − Recovery studies.		
Concentration (μg/mL)	Calculated concentration (μg/mL) ± SD; RSD% ($n = 3$)	Recovery (%)
10	9.76 ± 0.16; 1.681	97.6
50	48.97 ± 0.38; 0.768	97.9
75	76.20 ± 0.98; 1.291	101.6
RSD = relative standard deviation; SD = standard deviation.		

A

+Q1: 0.014 to 0.483 min from Sample 2 (0.2 ppm Epidepride Q1 scan 02) of 20090618 Epidepride Q1scan.wiff (Turbo Spray)   Max. 4.7e6 cps.

B

+MS2 (419.50) CE (30): 0.014 to 0.268 min from Sample 1 (0.2 ppm Epidepride MS2 of 419.5 01) of 20090618 Epidepride MS2 scan.wiff (Turbo ...   Max. 3.6e4 cps.
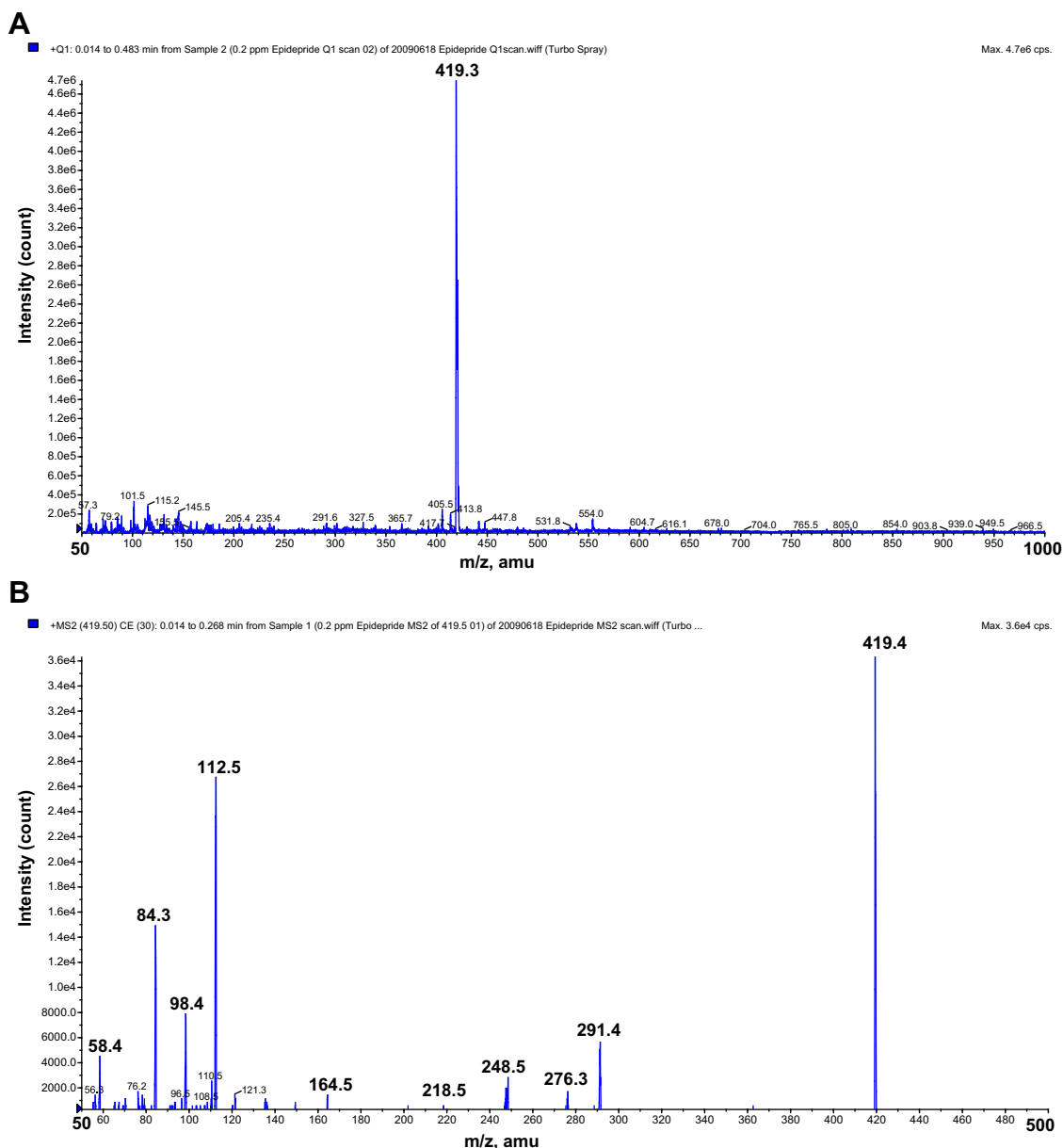
Fig. 4 – Mass spectra of epidepride: (A) Q1 full scan, (B) tandem mass spectrometry from m/z 419.

oxidation, and oven heating. The epidepride samples were dissolved in 0.33 mL methanol (3 mg/mL) and immersed in 0.33 mL 1 M HCl or 1 M NaOH at ambient temperature for 30 minutes, 1 hour, 2 hours, 8 hours, and 24 hours. The acidic and alkaline hydrolysis samples were neutralized using 0.33 mL 1 M NaOH or 1 M HCl, respectively, prior to HPLC analysis. The oxidation study was performed by mixing 0.5 mL of 2 mg/mL epidepride solution with 0.5 mL 3% H_2O_2 and maintaining the mixture at room temperature for 30 minutes, 1 hour, 2 hours, 8 hours, and 24 hours. For the thermal stress studies, about 1 mg of the drug was sealed in glass vials and placed in an oven at 50°C for the same times mentioned above [19,20]. After incubation the samples were diluted into 10 times volume in methanol for HPLC–PDA analysis.

3.   Results and discussion

3.1.   HPLC method development

A reversed-phase HPLC method was developed to study epidepride. The ligand was analyzed on a monolithic silica with a characteristic porous structure, whereas surface modified with C-18 and a fully end-caping column using a 15:85 methanol–acetonitrile mixture, and ammonium acetate (10 mM, pH 7.0) was added to both solvents. The absorption spectra of epidepride were recorded between 200 nm and 300 nm using a post-column PDA. The detection wavelength was set at 210 nm for determination of epidepride (including decomposed products). A typical HPLC chromatogram

Table 4 − Optimized mass spectrometry parameters for linearity studies of the MRM transitions of ^{127}I-epidepride.[a]

MRM transitions	DP	EP	CE	CXP	Linear regression eq.	Corr. coeff. (r^2)
419.4/291.4	100	10	41	15	$y = 5.70 \times 10^3 x - 4.35 \times 10^3$	0.9970
419.4/276.3			55	16	$y = 193 \times x - 168$	0.9987
419.4/248.5			60	14	$y = 1.94 \times 10^3 x - 2.60 \times 10^3$	0.9991
419.4/112.5			35	4	$y = 3.94 \times 10^3 x - 4.24 \times 10^3$	0.9992
419.4/98.4			55	6	$y = 2.25 \times 10^3 x - 2.01 \times 10^3$	0.9993
419.4/84.3			70	2	$y = 356 \times x - 233$	0.9993
419.4/58.4			65	2	$y = 768 \times x - 583$	0.9995

CE = collision energy; Corr. coeff. = correlation coefficient; CXP = collision cell exit potential; DP = declustering potential; EP = entrance potential; eq. = equation; MRM = multiple reaction monitoring.
[a] Dynamic range: 1−100 ng/mL.

obtained for epidepride is shown in Fig. 3 [retention time = (2.37 ± 0.01) minutes, RSD = 0.44%, $n = 18$].

3.2. Linearity

With the HPLC method described above, the calibration curve (with six concentration points) for epidepride was sufficiently linear in the concentration range between 1 μg/mL and 100 μg/mL. The linear least-square regression equation was $y = 20.26 \chi -22.54$, with a correlation coefficient (r^2) of 0.999.

The limit of detection and limit of quantification for the ligand were 0.02 μg/mL and 0.1 μg/mL, respectively.

3.3. Precision

The data for the intra- and inter-day precision studies, carried out within the linearity range at three different concentrations (10 μg/mL, 50 μg/mL, and 75 μg/mL), are shown in Table 2. The RSD values for the intra-day and inter-day precision study were <2%, which confirmed that the method was precise.
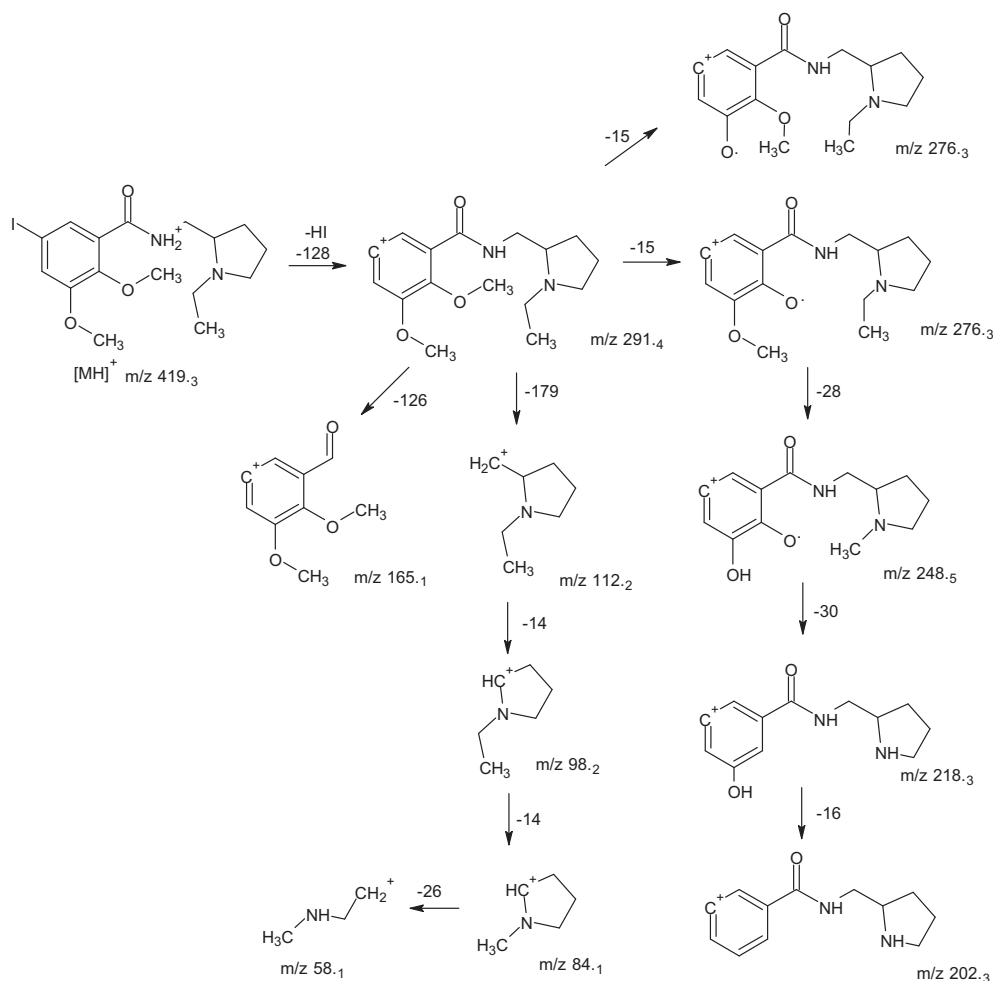
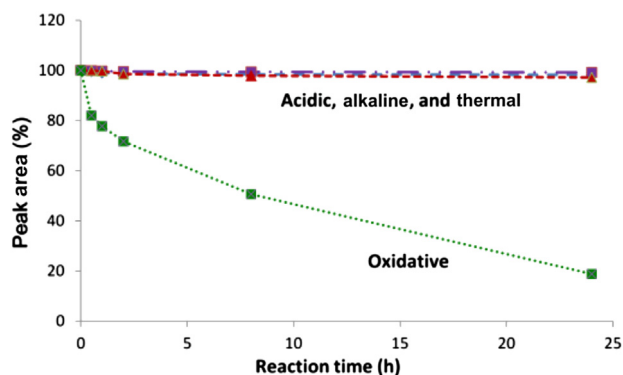Fig. 5 − Proposed fragmentation pathways of epidepride.

Fig. 6 – Transition trends of the forced degradation studies.

3.4. Accuracy

The percentage recovery was calculated by interpolating the peak areas of the spiked samples to the linear regression equation of the calibration curve. As shown in Table 3, good recoveries were obtained (95–105%), indicating that the developed method was sufficiently accurate. The validation results of the developed HPLC method regarding linearity, precision, and accuracy for epidepride measurements conformed well with the criteria listed in the guidelines issued by the ICH [16].

3.5. MS analysis of epidepride

MS analyses of epidepride fragments were initially performed by syringe infusion to introduce the sample solution into the equipment for Q1 and MS/MS scans. Full Q1 spectra were achieved in the negative-ion and positive-ion modes, but the latter mode was preferred because it gave more structural information about epidepride. In the electrospray ionization

spectra of epidepride, $[M + H]^+$ ions were observed at an m/z value of 419.3 (Fig. 4A). Product-ion scans were carried out at different collision-activated dissociation conditions to optimize the declustering potential (DP), entrance potential, collision energy, and collision cell exit potential. The production-ions of 419 were observed at m/z value of 291.4, 276.3, 248.5, 112.5, 98.3, 84.5, and 58.4 (Fig. 4B). LC-MS MRM transitions were also studied and are given, together with the optimized MS parameters, in Table 4, where the linear least-square regression equations and the correlation coefficients are also listed. The drug response was strictly linear, with all the investigated MRM transitions over the calibration range ($r^2 \geq 0.998$). The best quantification equation for the MRM transition was 419/112, with a highly sensitive slope and linearity across the concentration range between 1 ng/mL and 100 ng/mL. The limit of detection and limit of quantification for epidepride using MRM (419/112) were 0.01 ng/mL and 0.05 ng/mL, respectively. The others MRM pairs were used as criteria for identification in complicated-matrix specimen. Based on the performance of the established LC-MS/MS MRM method, we will investigate the bio-distribution (Bio-D) issue of epidepride from animal experiments in the future after the bioanalytical method is validated.

MS/MS fragmentation ions and a fragmentation pathway of epidepride are proposed on the basis of the MS/MS spectra in Fig. 5. The C–I bond could be easily cracked, because iodide is a good leaving group. The methyl groups of methoxybenzyl in the structure of epidepride were the second most easily cracked bonds, because the oxygen radicals are delocalized around the benzene ring.

3.6. Degradation behavior

Primarily, no epidepride degradation products were observed under acidic/alkaline hydrolysis and thermal decomposition conditions, although the retention time of the parent drug
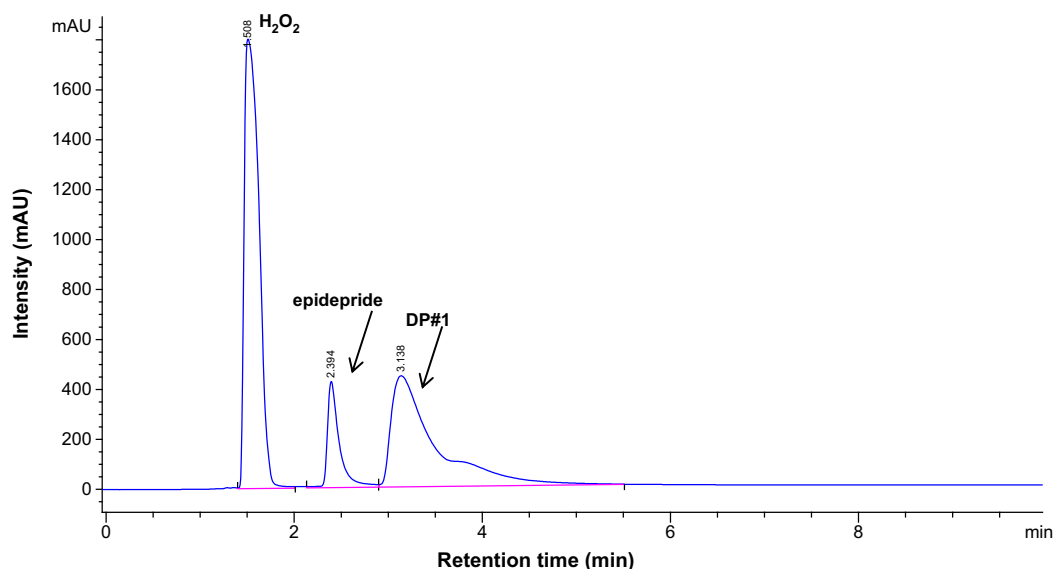

Fig. 7 – High-performance liquid chromatography of epidepride after being subjected to oxidative conditions for 24 hours. DP = degenerated product.
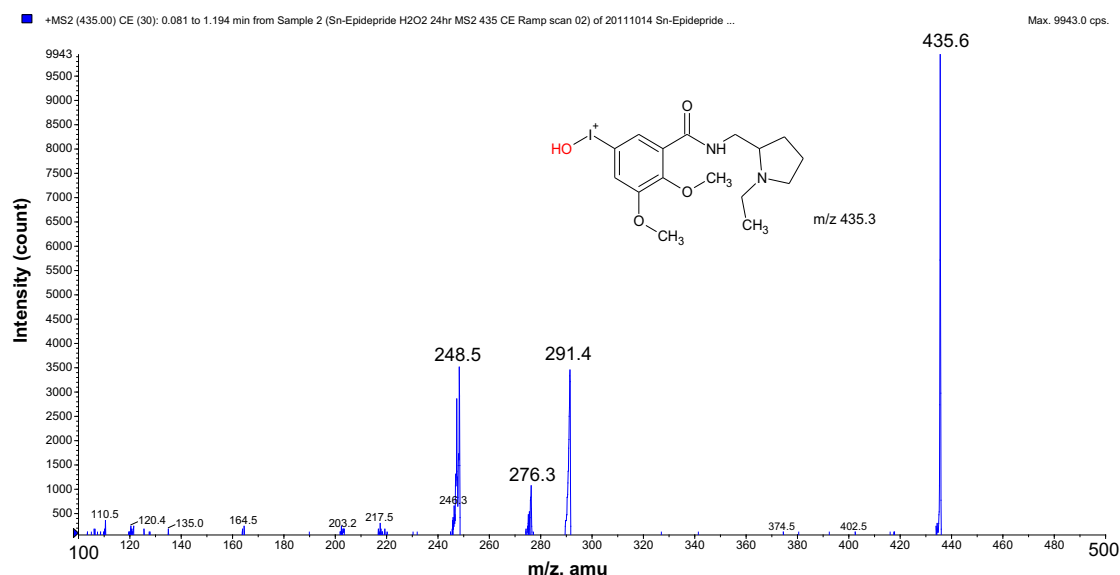
Fig. 8 – Product-ion spectrum of DP#1 with *m/z* 435 and its possible structure. DP = degenerated product.

shifted owing to changes in the ionic strength of the sample solutions. The ligand was relatively stable under such harsh conditions, and the trends of the forced degradation studies are plotted in Fig. 6. The chromatographic purities of epidepride, obtained from HPLC studies after acidic, alkaline, and thermal stress testing of the sample, were >97.5%, despite incubation for 24 hours. However, >80% of the original amount of epidepride deteriorated within 24 hours under oxidative conditions, and a degenerated product peak (DP#1, t_R 3.13 min) appeared and the peak area increased gradually after the beginning of the reaction for 30 minutes. A chromatogram of the oxidation product, obtained after 24 hours, is shown in Fig. 7. The *m/z* value of epidepride is 419, but we found (through a Q1 scan by LC–MS) that the degenerated epidepride product DP#1 had an *m/z* value of 435. The tandem mass spectrum of product-ions from the *m/z* 435 is shown in Fig. 8, and all the product-ions observed in this spectrum also existed as fragments of epidepride. It could thus be demonstrated that DP#1 originated from epidepride; therefore, we believe that DP#1 should be an oxidation product of epidepride. A possible structure for DP#1 is illustrated in Fig. 8, which shows that iodo-phenyl is oxidized into hydroxy-iodo-phenyl, instead of the core structure of epidepride being oxidized, so that does not shift the *m/z* values of fragments from epidepride. The proposed structure of DP#1 originates from the character of iodine with multi-oxidation-state bonding. The results also suggest that iodo-phenyl bonding is an active site to undergo various reactions.

4. Conclusions

HPLC and LC–MS/MS methods for epidepride determination were developed and verified. A series of stress studies was conducted on the ligand based on the HPLC and LC–MS analysis method, showing that epidepride is stable under thermal, acidic, and alkaline hydrolysis conditions but

deteriorates easily in an oxidative medium. The fragmentation pathways of epidepride and a possible oxidized structure were also proposed, and the iodide bond could be cracked and oxidized easily. The results of this study suggest that the drug should be stable within a wide pH range under ambient temperature conditions (with a long shelf life), but must be protected from oxidizing agents, and it may become a hydroxyl-iodo compound. Understanding the fragmentation pathways of epidepride could also help us to determine possible metabolites structures from LC–MS/MS studies on animal models in the future.

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