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Structural Analysis of Reserpine Degradation Products by LCMS-IT-TOF

Technical Report vol.20

1. Introduction

Structural elucidation of impurities during the development of pharmaceutical products is very important. While NMR and other techniques can be used for this purpose, the mass spectrometer is the primary means of conducting structural analysis. In order to do this, high mass accuracy with MS^n fragmentation data is absolutely necessary.

The acid degradation products of reserpine were used as model reaction products and impurities of pharmaceutical products, and were analyzed using an LCMS-IT-TOF mass spectrometer for the analysis. The acquired MS^n data were examined using analytical software including composition formula prediction software and MetID Solution software for determining the compositions and predicting the structures of the degradation products. The structural similarity search function of MetID Solution was used not only for metabolite analysis, but for searching and conducting structural analysis of impurities as well. (See Technical Report vol. 16)

* Data were acquired with the cooperation of Toray Research Center, Inc.

2. Method

Preparation of acid degradation products of reserpine: 1 mL methanol and 1 mL 1N-HCl were added to 100 mg reserpine (Tokyo Chemical Industry Co., Ltd.) and the decomposition reaction continued for 3 hours at 100 deg. C. The reaction solution was then diluted 100-fold for use as the test solution.

Analysis: The HPLC used was the Shimadzu Prominence system, and the mass spectrometer was the Shimadzu LCMS-IT-TOF. The LCMS-IT-TOF was used in the auto MS^n mode to automatically select precursor ions. The details are as follows.

Column :	ODS column 2.0mm I.D. x 50 mmL
Mobile phase A :	5mmol/L ammonium formate - water
Mobile phase B :	acetonitrile
Gradient program :	20%B (0 min) → 80%B (15 min)
Flow rate :	0.2 mL/min
Injection volume :	1 uL
Column temperature :	40 deg. C
Ionization mode :	ESI (+)
Nebulizing gas :	1.5 L/min
Drying gas pressure :	100 kPa
Probe voltage :	+4.5 kV
CDL temperature :	200 deg. C
BH temperature :	200 deg. C

3. Results

Fig. 1 shows the total ion chromatogram (TIC) and the $[M+H]^+$ mass chromatograms of the 10 major compounds shown in the TIC.

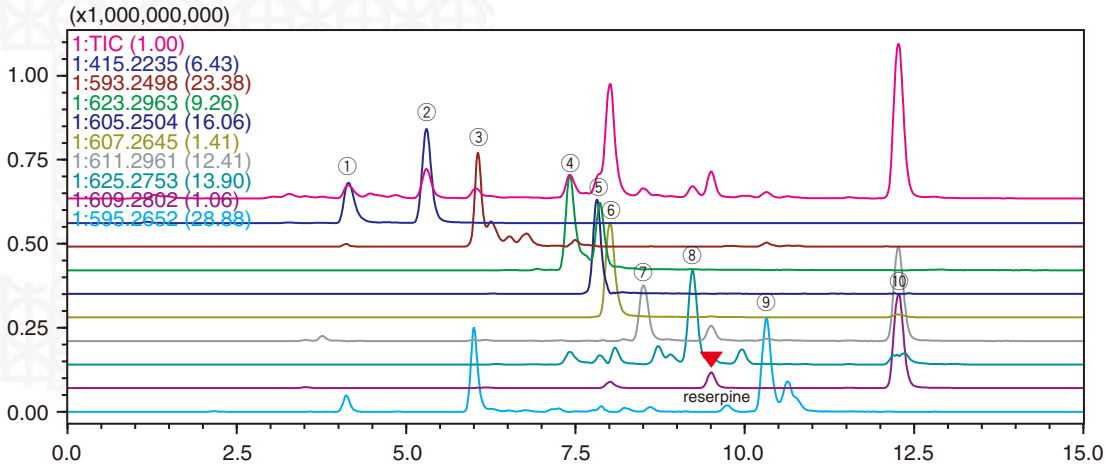


Fig. 1: Mass Chromatograms of the Acid Degradation Products of Reserpine

Fig. 2 shows the MS^2 analysis mass spectrum of reserpine (m/z 609.2802 was selected as the precursor ion). The cleavage sites in reserpine were predicted based on the fragmentation ion information obtained from MS^2 analysis and MS^3 analysis, and the structural formulas for these are indicated in Fig 3.

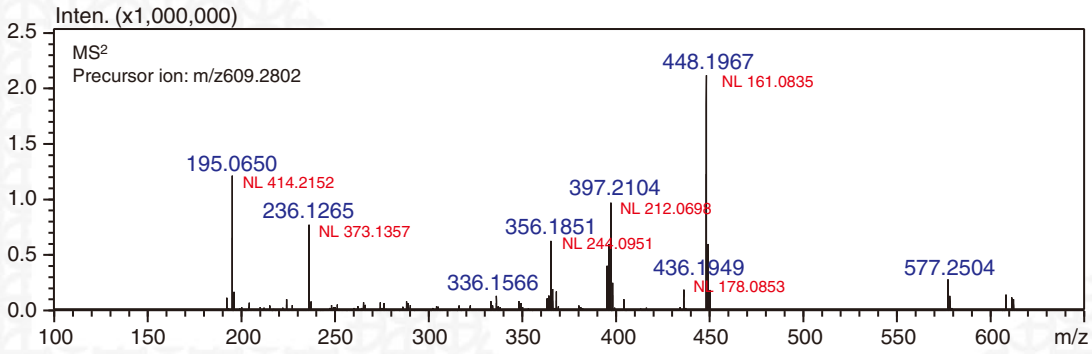


Fig. 2: Product Ion Spectrum of Reserpine (NL: Neutral Loss)

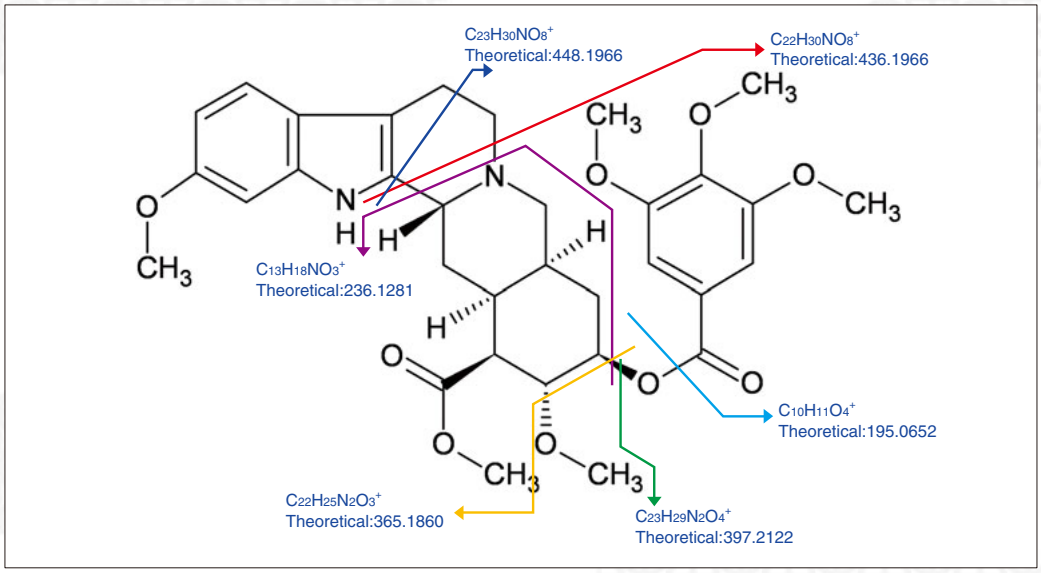


Fig. 3: Assignment of the Major Product Ions of Reserpine

The obtained data file was processed using MetID Solution. Fig. 4 shows the results screen.
MetID Solution was used to extract the product ions and neutral loss constituents for reserpine (Fig. 4a). Those product ions and neutral loss constituents which have common components with reserpine can be viewed in the extended results screen (Fig. 4b).

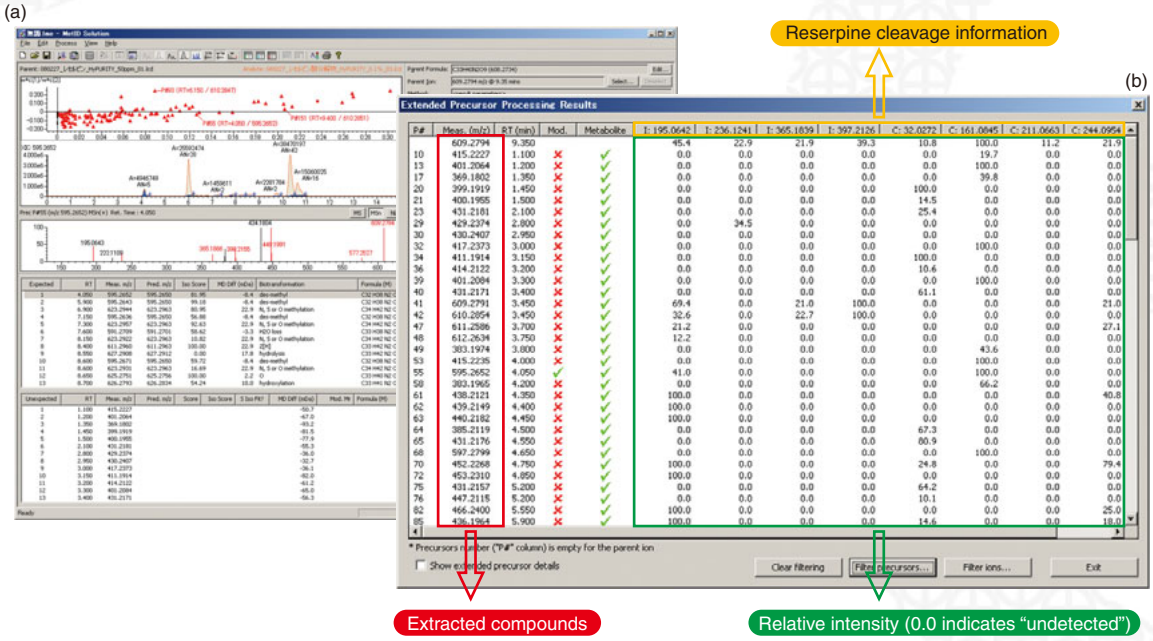
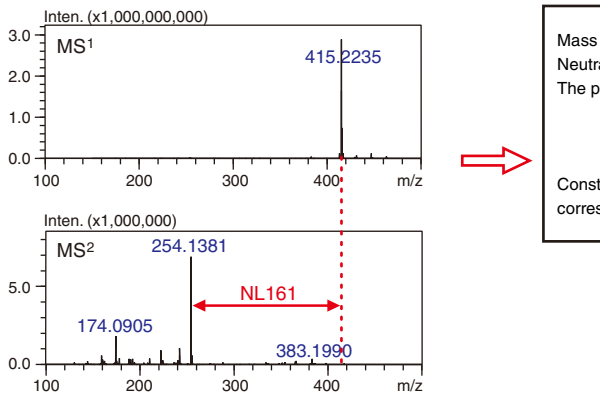


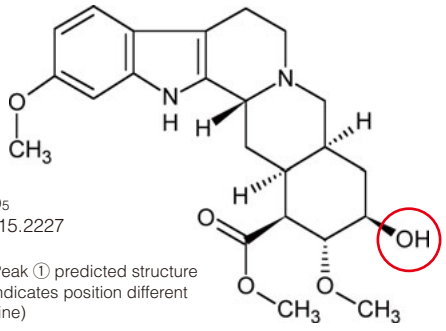
Fig. 4: MetID Solution Screenshots (a) Calculated Results of Product Ion Spectra - The listed compounds are common cleavage products from reserpine or have common substructures with reserpine.
(b) Table of Precursor Ions with Common Product Ions and Neutral Loss with Reserpine

Peak ① (m/z 415) and peak ③ (m/z 593) of Fig. 1 are described in detail as an example of structural analysis of reserpine degradation products. The compositional formulas that correspond to the mass differences between reserpine and peak ① and peak ③, respectively, were determined using the composition prediction software.



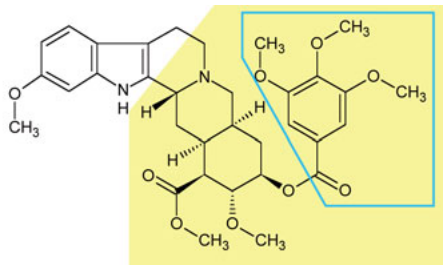
Mass difference with respect to reserpine = -194.0567 Da … $C_{10}H_{10}O_4$ (theoretical value 194.0579)
Neutral loss in common with reserpine … 161.08 (see Fig. 2)
The product ion that gives rise to neutral loss 161 is m/z 448 in reserpine. (see Fig. 3)

Constituent peak ① contains the yellow-colored component which has a structure (m/z 448) corresponding to a difference with respect to reserpine. That mass difference is -194 Da ($C_{10}H_{10}O_4$).



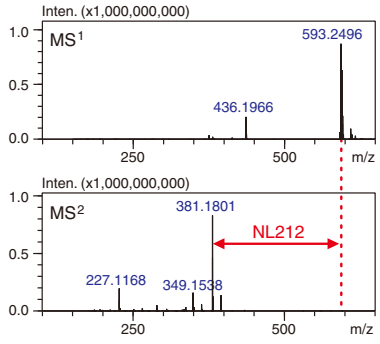
$C_{23}H_{30}N_2O_5$
$[M+H]^+$: 415.2227

○Example of Peak ① predicted structure ('red circle' indicates position different from reserpine)



The structure corresponding to the mass difference of 194 Da ($C_{10}H_{11}O_4$) within reserpine is indicated in the blue enclosure. That structure is predicted to exist within the yellow-colored structure (m/z 448).

Fig. 5: The Procedures of Structural Elucidation for Peak No. 1

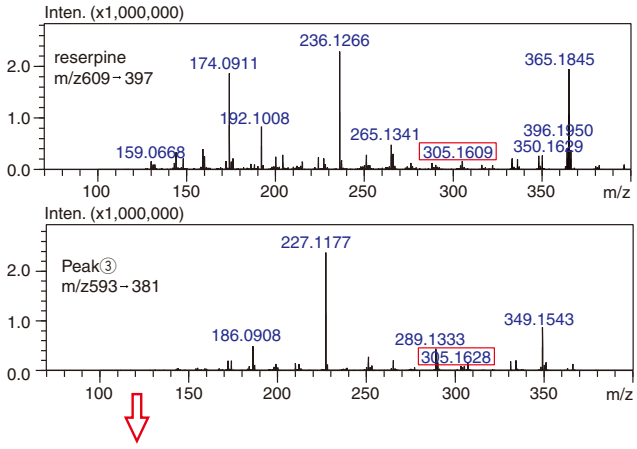


Mass difference with respect to reserpine = -16.0304 Da … CH_4 (theoretical value 16.0313)
Neutral loss in common with reserpine … 212.07 (see Fig. 2)
The product ion that gives rise to neutral loss 212 is m/z397 in reserpine. (see Fig. 3)

↓

Constituent peak ③ contains the yellow-indicated component which has a structure (m/z 397) corresponding to a difference with respect to reserpine. That mass difference is -16 Da (CH_4).

↓ MS^3 analysis of ion that gives rise to common neutral loss 212



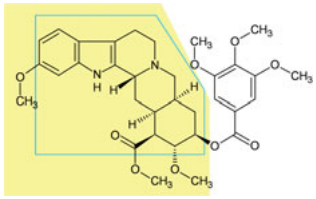
↓

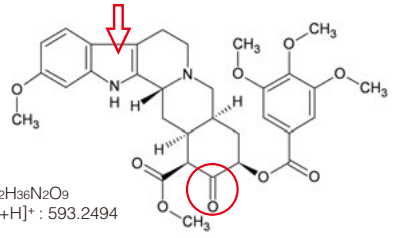
Fragment ion in common with reserpine and Peak ③ … m/z 305.16

↓

There is a difference of -16 Da (CH_4) at positions other than the structure of m/z 305.
= 3 locations of methoxy radicals, one of which is demethylated.

The structure corresponding to the mass difference of 305 Da ($C_{20}H_{21}N_2O_4^+$) within reserpine is indicated in the blue enclosure. That structure is predicted to exist within the yellow-indicated structure (m/z 397).





$C_{32}H_{36}N_2O_9$
$[M+H]^+$: 593.2494

○ Example of Peak ③ predicted structure
('red circle' indicates position different from reserpine)

As shown in the analysis examples of peaks ① and ③, high accuracy MS^n measurement is a useful tool for structural analysis of degradation products. Using the MS^3 analysis results for peak ③, the structure was predicted very accurately.
In this way, use of the LCMS-IT-TOF in conjunction with analysis support software including MetID Solution can greatly shorten the time required for structural analysis. Therefore, it is expected that this combination can help speed up the development process of bringing final pharmaceutical products to market.
Regarding the other degradation products, Peaks ① and ③ were also analyzed, and the results are shown below.

	Peak ①	Peak ②	Peak ③	Peak ④	Peak ⑤	Peak ⑥	Peak ⑦	Peak ⑧	Peak ⑨	Peak ⑩
Precursor ion (m/z)	415.2235	415.2238	593.2498	623.2963	605.2504	607.2645	611.2961	625.2753	595.2652	609.2797
Predicted composition	$C_{23}H_{30}N_2O_5$	$C_{23}H_{30}N_2O_5$	$C_{32}H_{36}N_2O_9$	$C_{34}H_{42}N_2O_9$	$C_{33}H_{36}N_2O_9$	$C_{33}H_{38}N_2O_9$	$C_{33}H_{42}N_2O_9$	$C_{33}H_{40}N_2O_9$	$C_{32}H_{38}N_2O_9$	$C_{33}H_{40}N_2O_9$
Variance with theoretical value (ppm)	1.93	2.65	0.67	0	1.65	-0.82	-0.33	-0.48	0.34	-1.64
Mass difference with respect to reserpine	-194.0567	-194.0564	-16.0304	+14.0161	-4.0298	-2.0157	+2.0159	+15.9951	+14.015	0
Composition indicated by difference	$C_{10}H_{10}O_4$	$C_{10}H_{10}O_4$	CH_2	CH_2	H_4	H_2	H_2	O	CH_2	0
Predicted existence position of difference	Within m/z448	Within m/z448	Within m/z397 Out of m/z305	Within m/z365	Within m/z397	Within m/z305	Within m/z236	Within m/z305	Out of m/z397	-

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