應用手冊

LC-MS Analysis of *trans*-Retanoic Acid

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Abstract

This application note demonstrates LC-MS analysis of *trans*-retinoic acid.

Introduction

The purpose of this study was to obtain the molecular weights for *trans*-retinoic acid by electrospray ionization. In addition, the characteristic API (atmospheric pressure ionization) fragmentation patterns were obtained.

Preliminary experiments indicated electrospray negative mode was the most efficient for ionizing retinoic acid. A single quadrupole Mass Spectrometer can be used for obtaining useful fragmentation patterns when the individual analytes are pure compounds or are physically separated by HPLC. The fragmentation is induced by collision-induced dissociation (CID). This happens when the sample cone voltage is raised.

Experimental

Experimental Conditions

LC-MS:	Waters Alliance System, 2690 Separations Module, Platform LC Mass Detector
Compound:	*trans*-Retinoic acid
Column:	No column for flow injection analysis (FIA) through a restrictor
Mobile phases:	50:50 Acetonitrile and 5 mM ammonium acetate isocratic blend
Flow rate:	0.2 mL/min
MS Detection:	ESI$^-$, scan 100–350 *m/z*
Fragmentation:	CID at cone voltages of 30, 50 and 60V

Results and Discussion

It was determined that retinoic acid was more responsive in the negative mode operating with the electrospray probe. All the analysis was performed by FIA (flow injection analysis) at 30 µL/min. The spectrum of retinoic acid illustrates the unit mass resolution of the compound $[M-H]^-$ at 299.1 from its two isotopes at, 300.1 and 301.0. The Waters Platform LC and the subsequent instruments, Platform LCZ, ZMD and ZQ all have this degree of mass resolution.

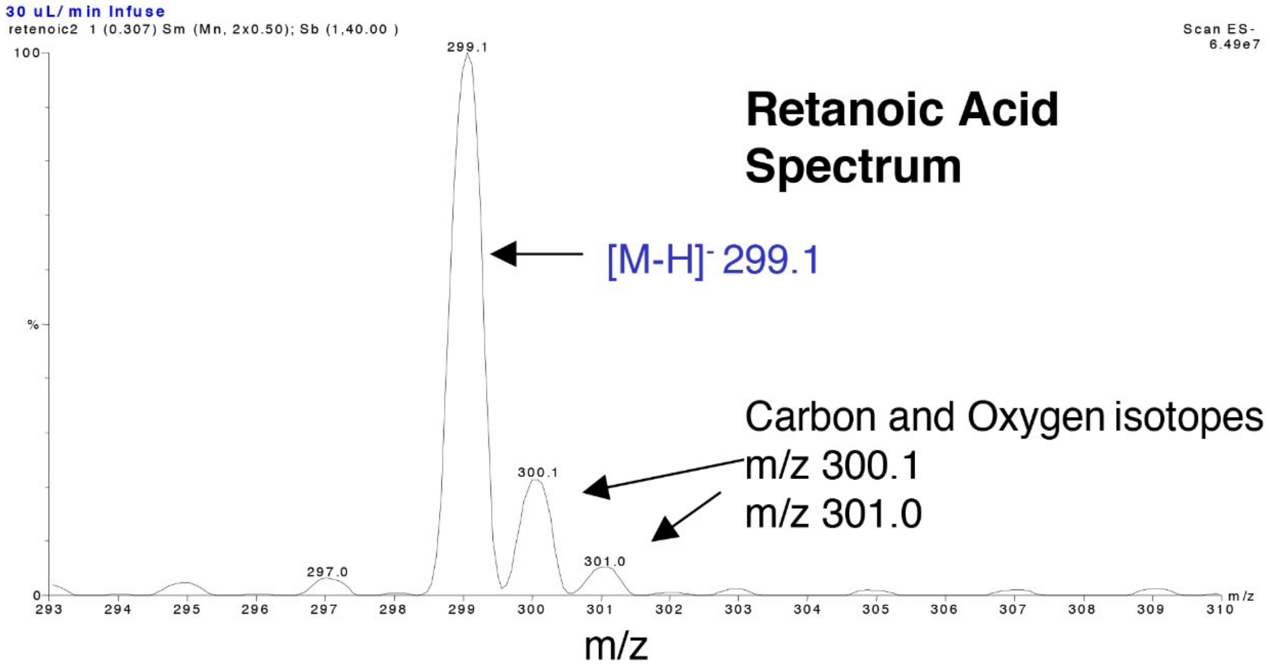

Figure 1. Mass Spectrum of retanoic acid.

The next stage of this investigation was to obtain API fragmentation information regarding retinoic acid. The Platform LC was setup to perform cone voltage switching at mass range of 100–350. One FIA experiment was performed with three voltages; 30V, 50V and 60V. By alternating cone voltage data at the three voltages are collected simultaneously.

Two flow injections are represented at the three cone voltages. Total ion chromatograms (TIC) were acquired. The TIC compiles the entire signal that was obtained within the mass spectral range. There are good signals at each cone voltage. Noise is reduced at the higher voltages.

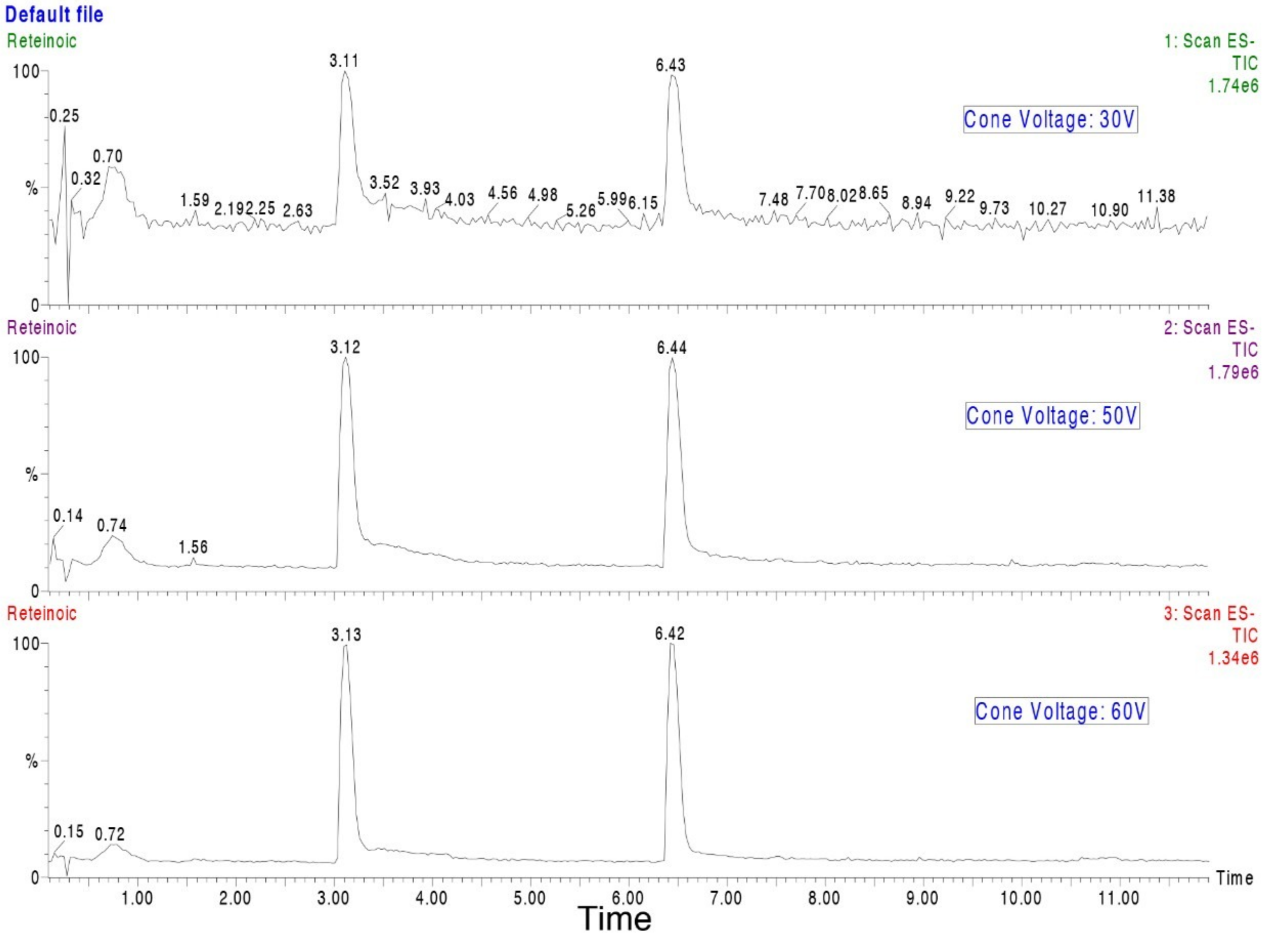

Figure 2. Flow Injection Analysis at Different Cone Voltages.

In Figure 3, the mass spectra were extracted for each of the cone voltages. The mass spectrum extracted at low cone voltage displays very little fragmentation of the parent ion $[M-H]^-$ 299.2 *m/z*. The proposed fragmention can be seen in the insert of the bottom panel. Collision-induced dissociation in a Single Quadrupole Mass Spectrometer can thus be used to confirm the identity of retinoic acid by the presence of the parent ion and a known fragment.

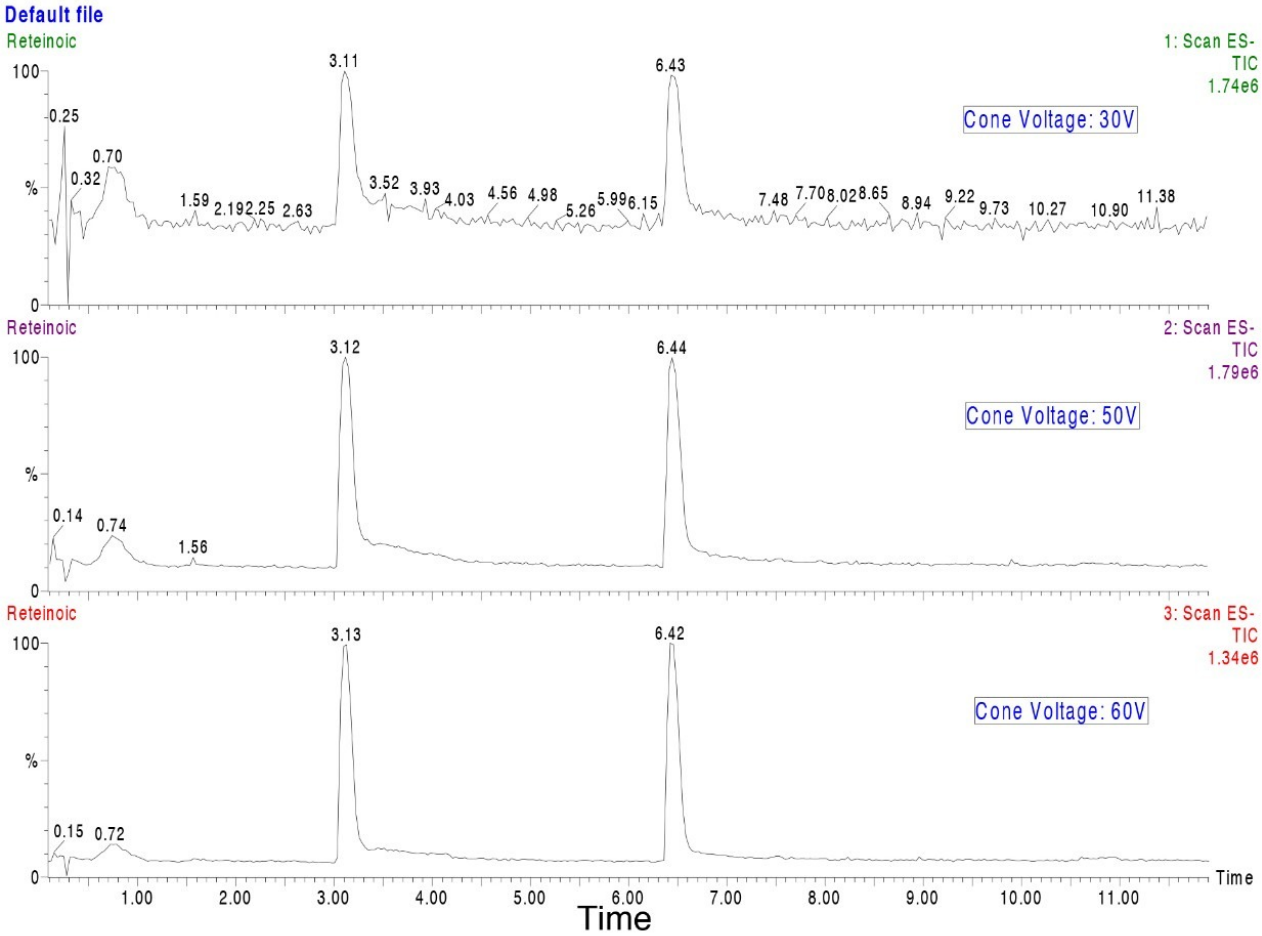

Figure 3. Mass Spectra at Different Cone Voltages.

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