Waters™

Note d'application

SYNAPT High Definition Mass Spectrometry System Analysis of Steroids

Jayne Kirk, Diana Uría

Waters Corporation

Abstract

To show the utility of high efficiency ion mobility based separations combined with high resolution tandem mass spectrometry for the structure elucidation of analytically challenging compounds, such as steroids.

Introduction

The structural elucidation of natural and synthetic compounds is key for many scientific developments. Identifying the chemical structure that confers special properties to a substance opens the door to a meaningful product design. The application areas can range from the development of new drugs to the discovery of new flavors for foods. This is no trivial task and usually requires a high degree of expertise by the analyst to elucidate the structure. The combination of several information-rich techniques, such as NMR, X-Ray Crystallography and mass spectrometry is usually required.

The normal MS approach typically starts with the proposal of elemental compositions. Using high resolution

mass spectrometry, mass accuracy and isotopic pattern information can be obtained. A second step, consisting of the acquisition of fragmentation data, is then performed. Fragmentation data contain structural information and enable the assignment of a chemical structure to the analyte. While this approach can be relatively straightforward for certain compounds, some compound classes are known for being especially reluctant to fragmentation.

This application note demonstrates the utility of the Waters SYNAPT High Definition Mass Spectrometry (HDMS) System, combined with MassFragment, a specific fragment prediction software package, for the structure elucidation of traditionally challenging compounds, such as steroids.

SYNAPT HDMS System.

Experimental

Sample Preparation

A methyl testosterone standard solution was prepared at a 1ng μL^{-1} level in a mixture of acetonitrile water (50:50 acidified with 0.1% of formic acid. The sample was infused at a rate of 5 µL/minutes.

MS Conditions

MS system:	SYNAPT HDMS System
Ionization Mode:	ESI +
Capillary Voltage:	3200 V
Cone Voltage:	30 V
Source Temp:	120 ˚C
Desolvation Temp:	200 ˚C
Desolvation Gas:	400 L/Hr
V-optics:	Enabled
IMS Pressure:	0.54 mbar
IMS wave:	5 to 15 V
Trap CE:	15 V
Transfer CE:	25 V

Mass range:	50-1000 *m/z*

Acquisition and Processing Methods

The data were acquired using Waters MassLynx Software, v. 4.1.

This data was processed using DriftScope Software. This software package is designed to interpret four-dimensional datasets containing the retention time, drift-time, *m/z*, and intensity obtained from the SYNAPT High Definition Mass Spectrometry System.

Results and Discussion

Fragmentation of steroids is frequently applied as part of a screening method, especially in regulatory analysis where confirmation of identity is generally based on three or four diagnostic product ions.[1] The identity of those diagnostic ions has been the subject of numerous scientific publications, mainly because of the complexity of the fragmentation reactions involved.[1,2]

When subjected to collision induced fragmentation (CID) at low collision energy, steroids tend to fragment by the loss of small neutral molecules and at one or two specific ring positions via chargemediated reactions.[2] The fragments created in this manner are not always specific enough to allow the unambiguous identification of the compound, as there can be several isomeric species for steroids. More energetic conditions tend to produce over-fragmentation of the steroids when only small non specific fragments are visible.

The SYNAPT HDMS System with its patented TriWave technology provides the ability to perform Time Aligned Parallel (TAP) fragmentation.[4] In TAP analysis, the ion of interest (selected by the quadrupole) is fragmented in the TRAP T-Wave. These first generation product ions are then separated in the IMS T-Wave using high-efficiency ion mobility (diagonal series of ions in Figure 1). The separated packets of ions are then subjected to a second stage of CID in the TRANSFER T-wave to produce second generation product ions (vertical series of ions in Figure 1). The second generation product ions share the same drift-time as their respective first generation precursors, which simplifies the interpretation of these data. TAP fragmentation provides comprehensive pseudo-MS3 information with high mass accuracy in a single experiment without the compromises in sensitivity

and accuracy associated with MS3 experiments on linear ion trap instruments.

By combining this information-rich technique with the excellent mass accuracy for both parent and fragments, along with structure elucidation software tools such as MassFragment, the user can achieve detailed structural information with high confidence in a single experiment.

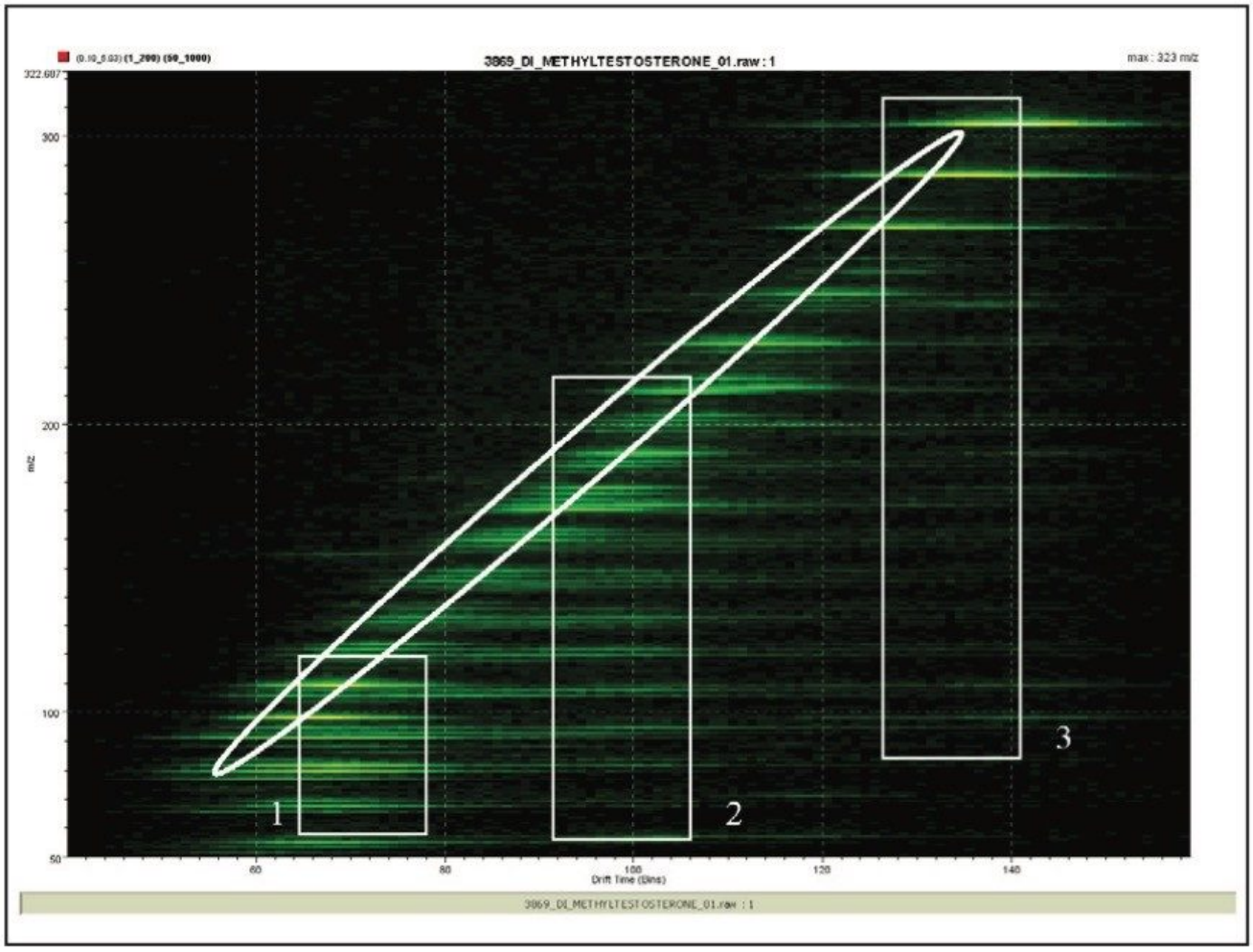
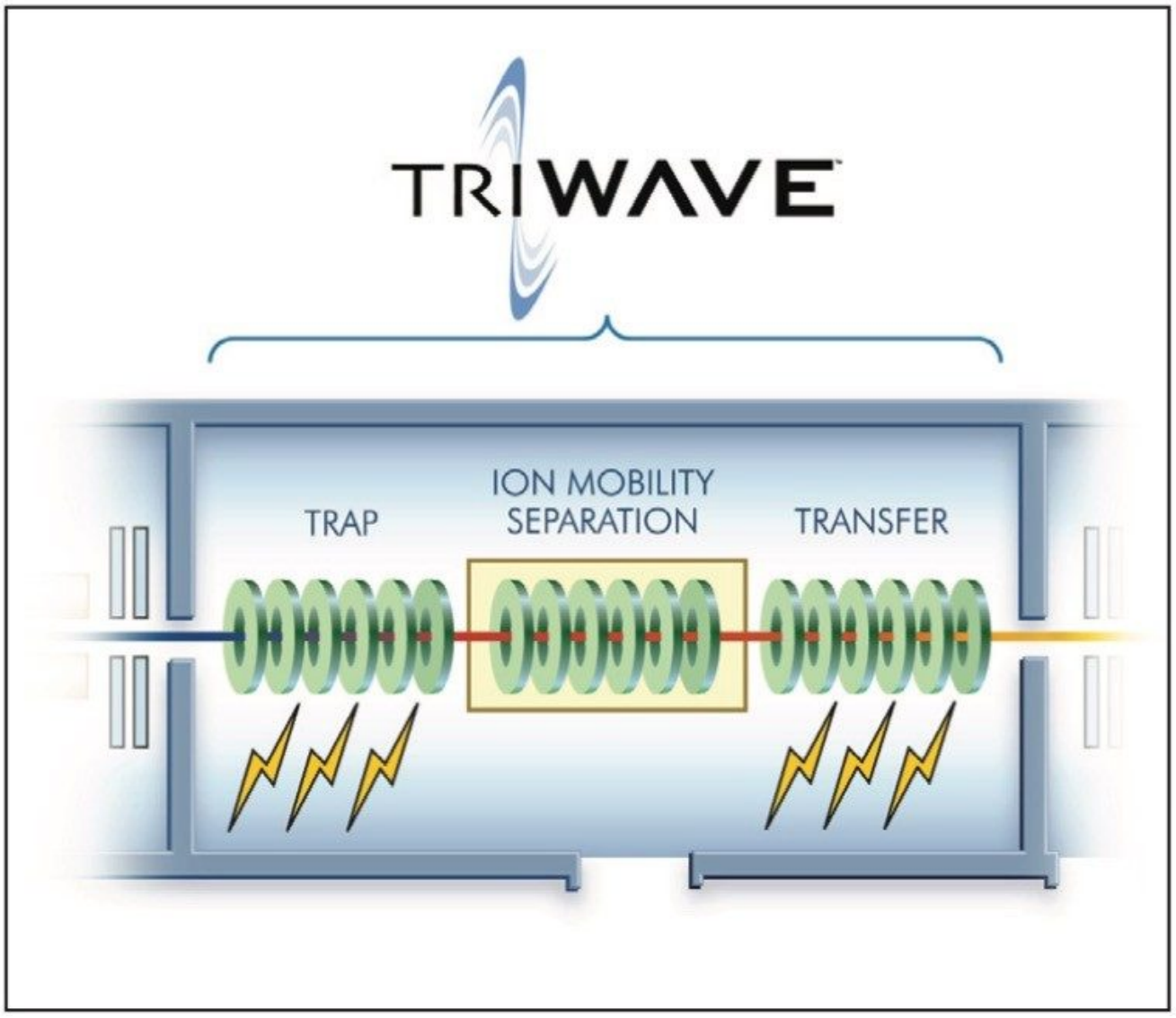


Figure 1. TAP fragmentation for methyl testosterone.



TriWave - the enabling technology within the SYNAPT HDMS System.

TAP Fragmentation Experiments on Methyl Testosterone

The *m/z* versus drift-time plot for the TAP fragmentation experiment of methyl testosterone shows clear regions of data related to the first and second generation of fragment ions (Figure 1). The signals on the diagonal contain information about the intensity and drifttime for the first generation products and the regions on the vertical axis contain the second generation fragments aligned with their respective precursor.

By selecting the appropriate regions and extracting the mass spectrometric information, independent (in Driftscope Software) MS/MS spectra can be reconstructed for methyl testosterone. Each of these spectra contains key information for distinctive structural regions. (Figure 2).

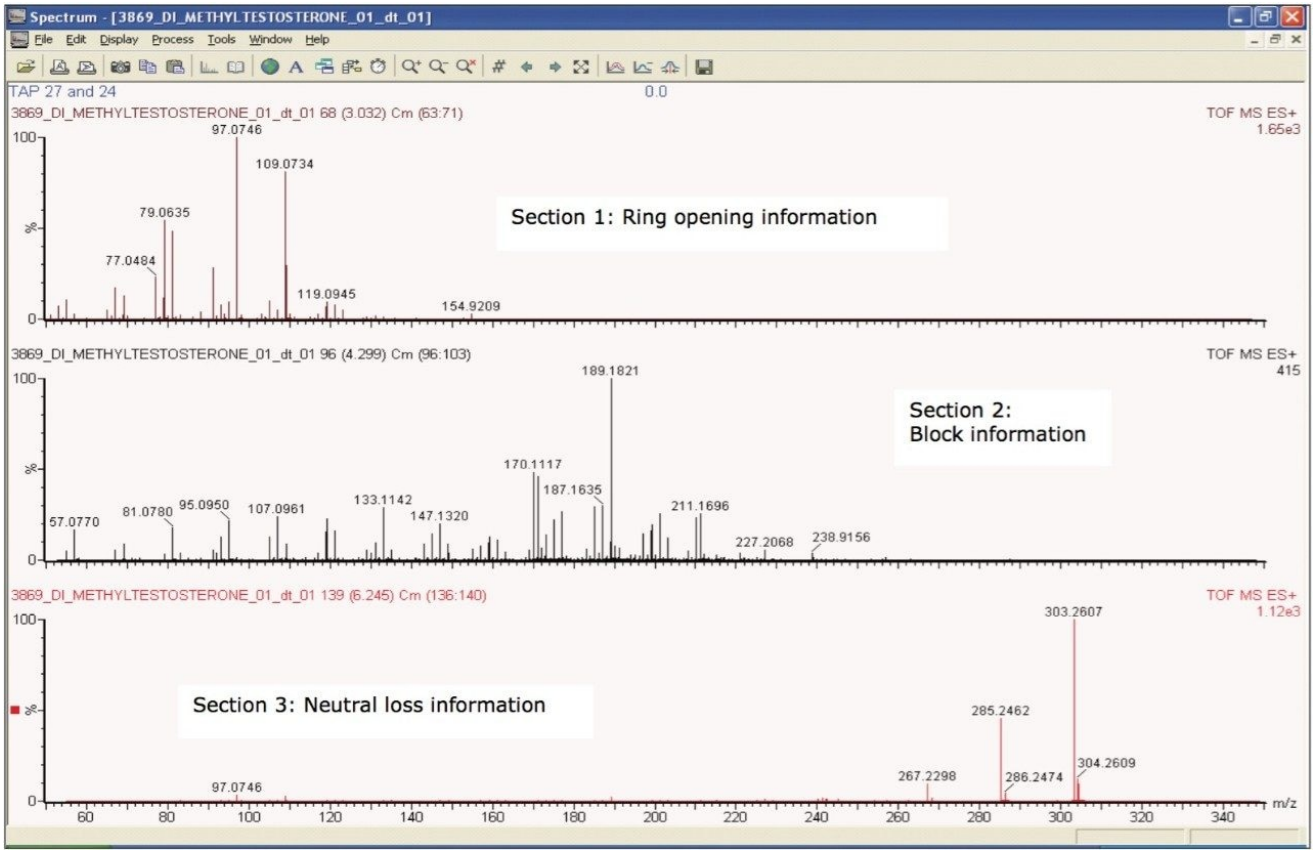


Figure 2. Specific MS/MS spectra for methyl testosterone.

The ions with longer drift-times that have been directly created from the precursor ion tend to reveal information about the possible neutral losses of the molecule. Water losses are especially easy to observe. The ions with shorter drift-times relate to the ring opening reactions and usually require high collision energy to be observed. The third region, corresponding to drift-times between 80 and 120 drift-time units relate to the sequential fragmenting of the assembly moieties of the steroid.

The mass accuracy provided by the SYNAPT HDMS System can be used for the proposal of elemental compositions for the fragment ions. The proposed results are summarized in Table 1. These calculations were performed by taking the elemental composition of methyl testosterone to limit the possible elements present in

the fragments and considering a maximum error of 3 ppm.

Empirical mass	Proposed elemental composition	Theoretical mass	Error (mDa)
303.2324	$C_{20}H_{31}O_2$	303.3024	0
285.2223	$C_{20}H_{29}O$	285.2218	0.5
267.2119	$C_{20}H_{27}$	267.2113	0.6
227.1802	$C_{17}H_{23}$	227.1800	0.3
215.1802	$C_{16}H_{23}$	215.1800	0.2
211.1487	$C_{16}H_{19}$	211.1487	0.1
201.1648	$C_{15}H_{21}$	201.1643	0.5
189.1649	$C_{14}H_{21}$	189.1643	0.6
185.1334	$C_{14}H_{17}$	185.1330	0.4
109.0653	C_7H_9O	109.0653	0
105.0708	C_8H_9	105.0704	0.4
97.0655	C_6H_9O	97.0653	0.2
91.0551	C_7H_7	91.0548	0.3
81.0709	C_6H_9	81.0709	0.5
9.0551	C_6H_7	79.0548	0.3
77.0398	C_6H_5	77.0391	0.7

Table1. Mass accuracy for the proposed elemental compositions.

MassFragment Software

Once Fragment data have been acquired, the characterization of fragment ions needs to be performed. The MassFragment Software tool, integrated within the MassLynx Software significantly aids this process by automatically identifying fragment ions using a series of novel, chemically-intelligent algorithms. MassFragment Software proposes structures to the observed fragment ions by assigning a score value to each proposed structure based on the likelihood of breaking certain types of bonds. The analyst confirms the most appropriate fragment structures based on their score assignment and number of hydrogen added or removed from the

structure and the exact mass error.

The proposed structures for several of the most intense fragment ions for methyl testosterone are displayed in Figure 3.

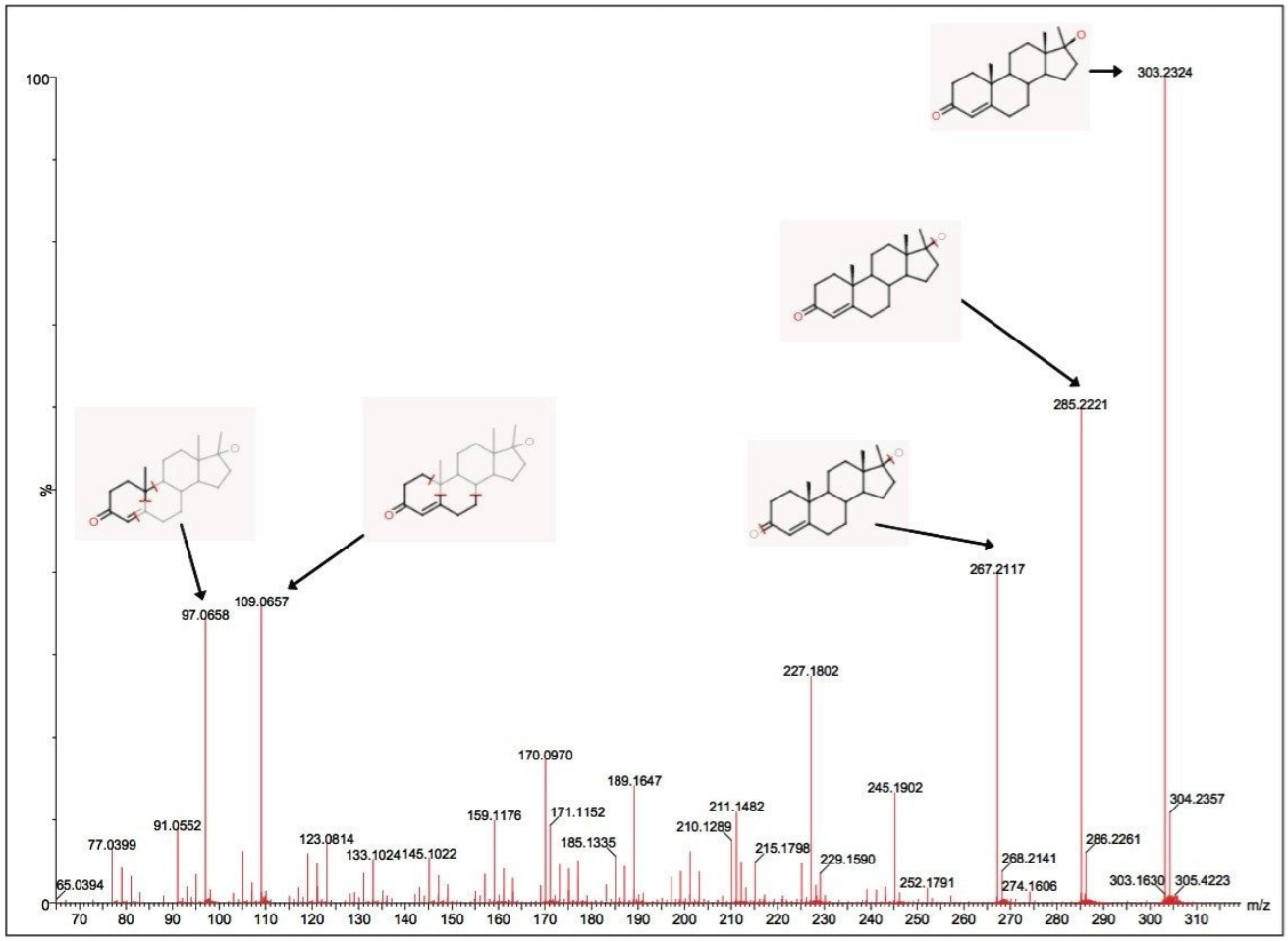


Figure 3. MS/MS spectrum for methyl testosterone with assignments proposed by MassFragment Software.

Conclusion

This works demonstrates that the SYNAPT HDMS System combined with the MassFragment Software package

provides a powerful platform to overcome complex analytical challenges. It offers a deeper understanding of the structural elucidation of natural and synthetic compounds and fragmentation pathways and proposes valid structures for the fragment ions. Even with traditionally challenging compounds, such as steroids this approach works provides a powerful, efficient route to comprehensive structural characterization.

This total Waters solution includes:

- SYNAPT HDMS
  - Extra dimension of separation and high-efficiency ion mobility separation
  - Time Aligned Parallel Fragmentation for advanced structural studies
  - Exact mass and isotopic pattern information that facilitates the successful identification of unknowns
- MassFragment Software
  - Chemically intelligent software tool for advanced fragment structure prediction

References

1. Williams, Kind, Houghton, and Hill. *J. Mass Spectrom*. 34, 2006–216, 1999.
2. Gua, Soma, and Luo. *J. Am. Soc. Mass Spectrom*. 17, 477–479, 2006.
3. Griffiths, *Mass Spectrom* Rev. 22, 81–152, 2003.
4. Yu, Castro-Perez, and Shockcor, Waters Application Note No. 720002542EN < https://www.waters.com/webassets/cms/library/docs/720002542en.pdf> .

Featured Products

MassLynx MS Software <https://www.waters.com/513662>

MassFragment <https://www.waters.com/1000943>

720002802, October 2008