

Small Scale Preparative Isolation of Corticosteroid Degradation Products Using Mass-Based Fraction Collection

Application

Pharmaceutical

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Abstract

The preparative isolation of corticosteroid degradation products using mass-based fraction collection is demonstrated. The results of the experiments done in this application note show that the Agilent Prep-C18 Scalar column, when combined with the Agilent Series 1100 mass-based fraction collection software, allows the successful isolation of purified fractions of degraded corticosteroids. The Agilent Prep-C18 Scalar columns used in this study provide consistent separations and sufficient capacity to produce enough sample to obtain high-quality mass spectra for each fraction. Mass-based fraction collection enabled a high degree of purity to be obtained, even under incomplete separation conditions.

Introduction

Corticosteroids are a family of drugs that include cortisol (hydrocortisone) and corticosterone, adrenal hormones found naturally in the body, as well as synthetic drugs such as triamcinolone and prednisone. HPLC represents a convenient, rapid and sensitive technique for the analysis and quantitation of these drugs. When investigating new synthetic or natural corticosteroids or attempting to identify trace impurities by large injections or degradation products, preparative quantities are needed.

In all production and storage of drugs, stability is an issue. Liquid formulations are more prone to degradation than are dry dosage forms. In commercial products various preservatives are used. In order to more easily study degradation, samples are dissolved in solvents without preservatives and then exposed to external influences, such as air, light, and heat, which can cause degradation reactions to occur. This study demonstrates the use of an Agilent Prep-C18 column to isolate and rechromatograph degradation products of the mixture of corticosteroids used in a previous study [1] and to demonstrate the ease and usefulness of mass spectroscopic-based fraction collection for the repetitive and reproducible isolation of degradation products.



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Methods

The structures of the corticosteroids used in this study are shown in Figure 1. The separation method, using an Agilent Prep-C18 Scalar column, was previously reported and is reproduced in Table 1 [1]. The same column was used here to separate the corticosteroids and their degradation products. The mixture used in the earlier study was allowed to sit for 2 months in direct sunlight. The clear glass container was periodically opened to allow the exchange of air in the vessel. This mixture consisted of the four corticosteroids dissolved in pure DMSO at a concentration of 50 mg/mL each. The analysis used an Agilent 1100 binary HPLC with degasser, autoinjector, column oven, and diode-array detector (DAD), attached to an

Agilent active splitter and fraction collector and to an Agilent liquid chromatography/mass selective detector (LC/MSD) system with Atmospheric Pressure Photo Ionization (APPI) spray chamber and source. The APPI was operated in positive scan mode: capillary inlet -3000 V, mass range 50–500 m/z with a variable fragmentor set at 70–220 V. An Agilent 1100 pump was used for makeup flow and to pump methanol/acetone (50/50) containing 0.1-mM ammonium acetate at 0.5 mL/min. The active splitter was set at a 1:25 split with the lower flow going to the LC/MSD and the higher flow to the fraction collector or to waste. Each peak collected was the result of five pooled runs which were then evaporated to dryness under nitrogen and redissolved in pure DMSO for reinjection.

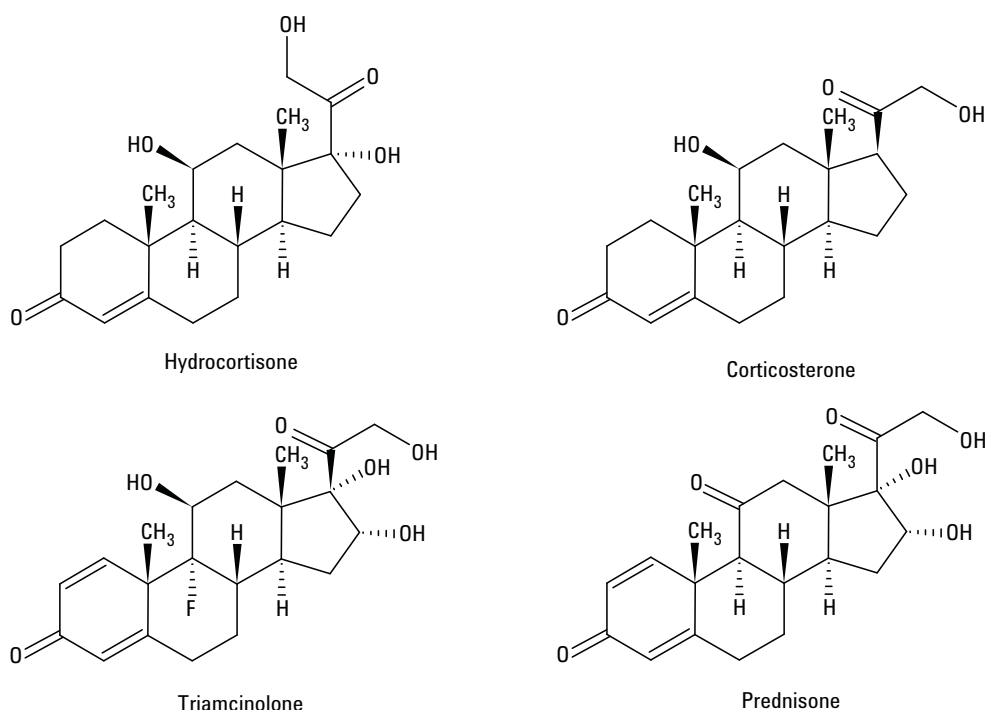


Figure 1. Structures of corticosteroids.

Table 1. Chromatographic Conditions for Separations of Corticosteroids

Column:	Agilent Prep-C18 Scalar Column, 4.6 × 150-mm, 10 μm
Mobile phase A:	Water
Mobile phase B:	50% Acetonitrile: 17% Methanol: 33% Isopropanol (v/v)
Gradient:	Time 0 min, 25% B ; 7 min, 25% B; 15 min, 34% B; 15.1 min, 45% B; 20 min, 45% B; 20.1 min, 25% B; stop time: 24 min
Flow rate:	1 mL/min
Injection solvent:	DMSO
Sample concentration:	Analytical runs: 1.5625 mg/mL; preparative runs: 50 mg/mL
Injection volume:	Analytical runs: 80 μL; preparative runs: 50 μL
DAD settings:	Detector wavelength: 244,8; Ref. 360,100
Flowcell:	0.06-mm pathlength
MS settings:	See text

Results and Discussion

The original separation [1] of the four corticosteroids is shown in Figure 2. The lack of degradation products in the chromatogram is obvious. This may be contrasted with the results shown in Figure 3, which show that additional peaks have appeared in this degraded sample. The upper trace is the DAD trace and the lower is the total ion

chromatogram (TIC) trace from the LC/MSD. The two degradation peaks chosen for isolation are noted on the TIC at principle ions of these peaks. The loading of sample for the small scale preparative runs in Figure 3 is 20X that of the analytical run in Figure 2. The loading was increased so that larger amounts of the degraded peaks could be collected for rechromatography.

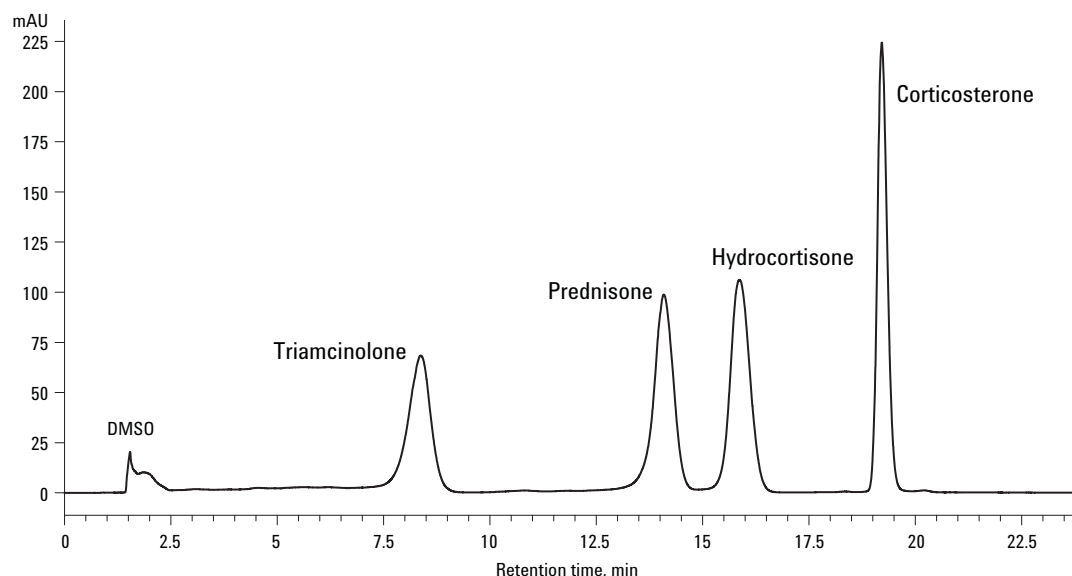


Figure 2. Analytical separation of corticosteroids on an Agilent Scalar-C18 Column.

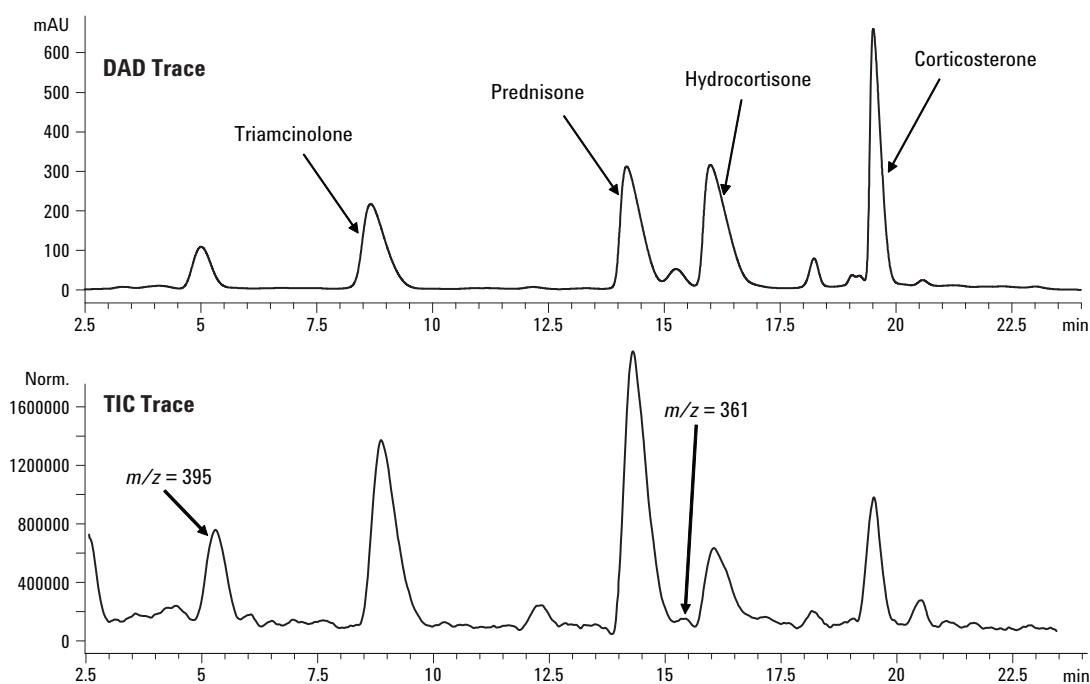


Figure 3. Degradation products of the four corticosteroids.

The principle goal of preparative chromatography is to isolate a pure component in as few runs as possible. As may be seen in Figure 3, due to resolution differences, these fractions are of varying degrees of difficulty to isolate in a relatively pure state. Figure 4 demonstrates a high degree of purity in the rechromatographed Fraction 1. This high purity was expected since Fraction 1 was well-resolved in the degraded mixture. This fraction can be considered “pure” as measured by the LC/MSD. From the presence of the m/z ion at 395, it can be deduced that the collected peak might be a degradation product from the corticosteroid triamcinolone, which also has an $m + 1$ ion at m/z 395.

It is common for preparative chromatography to be done under ambient temperature conditions. If time-based fraction collection is used, peaks can drift out of the collection window if the ambient temperature changes. Mass-based fraction collection minimizes this concern. It is only necessary to make one scouting run under scanning conditions and then reprocess the data to extract ions of interest. This initial run contains all the information needed to set up subsequent fraction collection runs. As may be seen in Figure 5, the isolation of Fraction 2 demonstrates the power of mass-based fraction collection. In order to collect Fraction 2, a small peak between two much larger peaks, it was only necessary to set a threshold above the inflection points between the peaks.

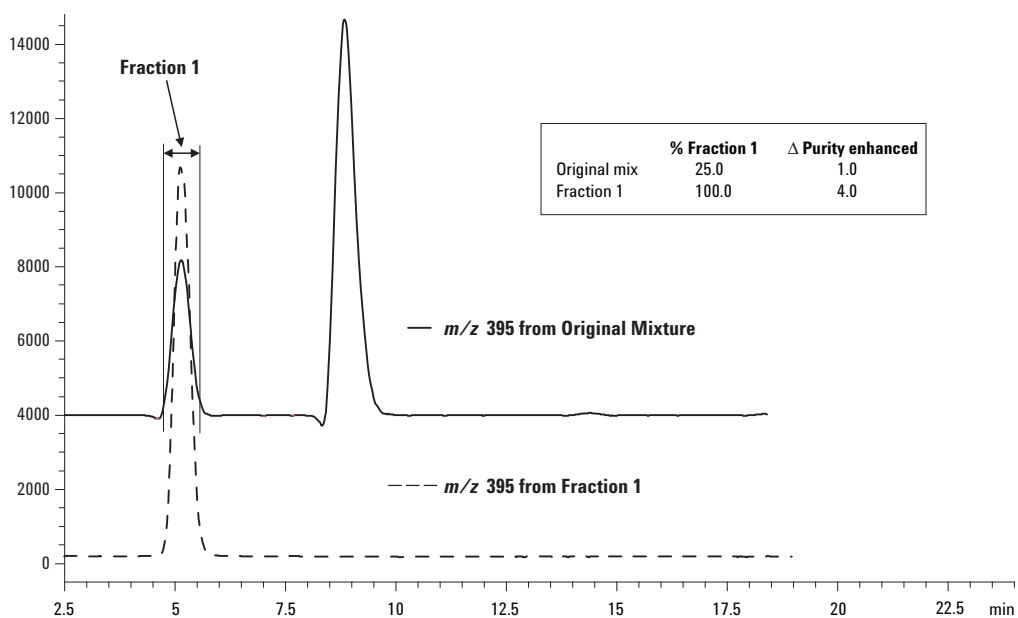


Figure 4. Rechromatography of Fraction 1.

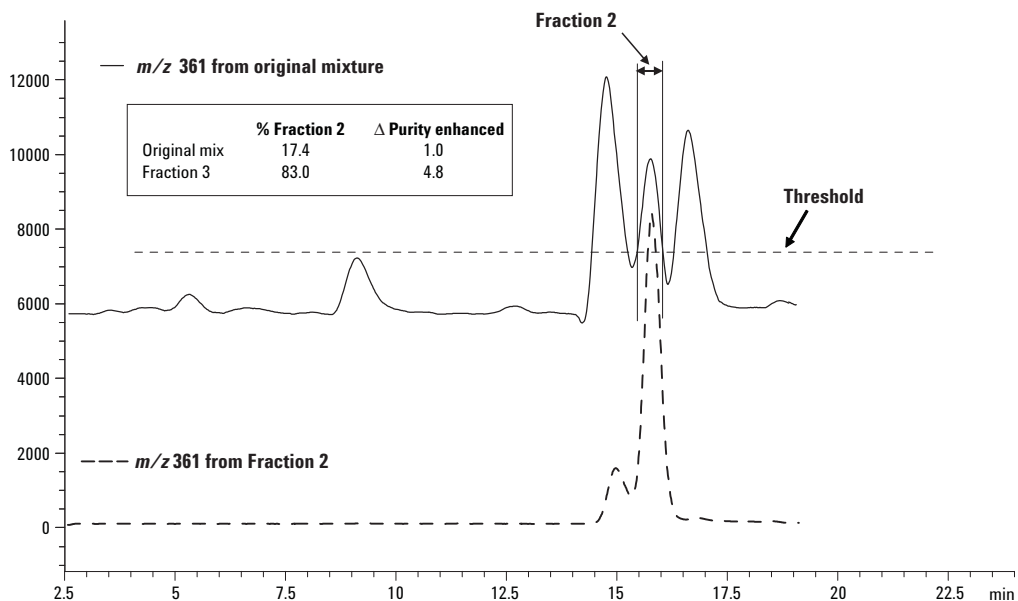


Figure 5. Rechromatography of Fraction 2.

It can be noted that Fraction 2 is not completely pure in this preparation. It is easy to see the cause - there is a much larger peak just before it, whose tail runs into it. This peak is tailing because the column is somewhat overloaded. Under analytical conditions, as seen in the rechromatography (Figure 5, lower trace), the two peaks are isolated enough to obtain clean mass spectra for the peak of interest. In order to obtain a completely pure isolation of Fraction 2 it would only be necessary to make more runs at a lower column load or to heart-cut and rechromatograph the collected fraction additional times.

The degradation products separated in these runs are each characterized by a unique mass spectrum. For example, if mass spectra of triamcinolone and

Fraction 1 are placed one above the other as in Figure 6, it is possible to see that they are related. There are two losses of m/z 20 ($395 \rightarrow 375$ and $347 \rightarrow 327$), characteristic of HF (a very common loss), and unique to this fluorine-containing steroid. There are also some losses of water in common (m/z $375 \rightarrow 357$ and $327 \rightarrow 309$). They share the same $M+H$ of 395. Triamcinolone also shows the loss of m/z 60 = CHOCH_2OH (m/z $395 \rightarrow 335$) characteristic of the neutral loss of the side chain at C17. Fraction 1 does not show this loss. The proportions of the various ions are also in very different ratios to each other in the two mass spectra. All of this adds up to Fraction 1 being a rearrangement of the molecular structure of triamcinolone, but with the same molecular weight.

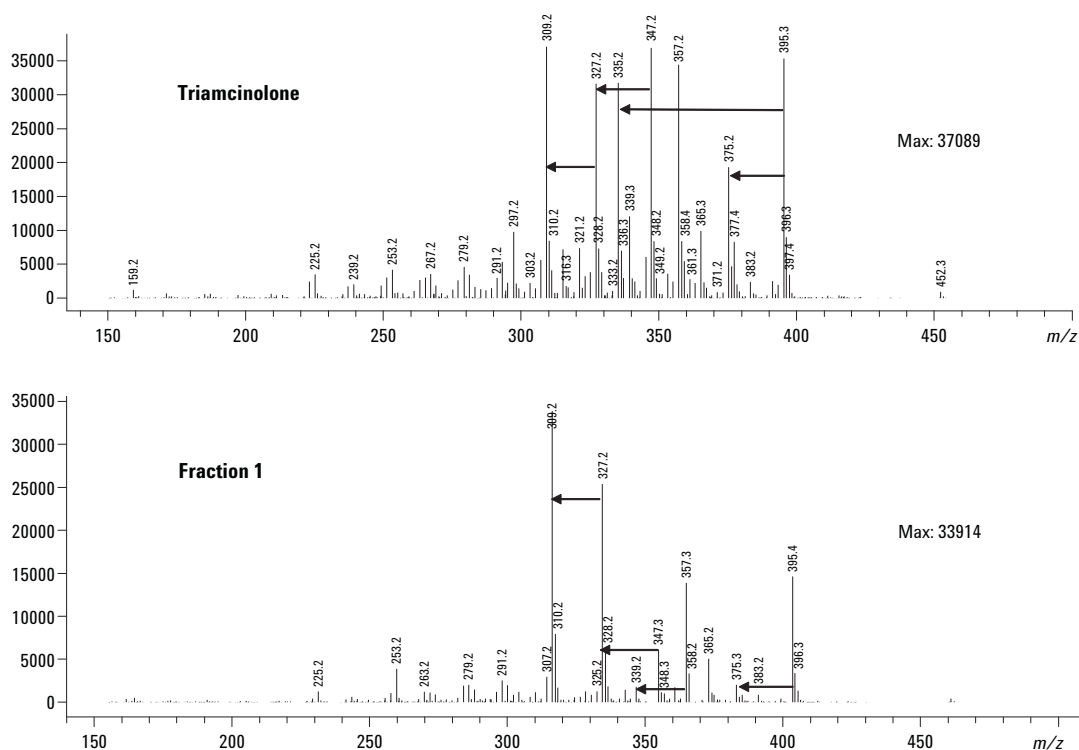


Figure 6. Mass spectra of triamcinolone and Fraction 1.

Conclusion

The results of this experiment show that the Agilent Prep-C18 Scalar column, when combined with the Series 1100 mass-based fraction collection software features, allows the successful isolation of purified fractions of degraded corticosteroids. The Agilent Prep-C18 Scalar column had sufficient capacity and reproducibility that only a few runs produced enough sample to obtain mass spectra of each fraction. Mass-based fraction collection enabled a high degree of purity to be obtained, even under incomplete separation conditions.

Reference

1. C. Woodward and R. Majors, "Preparative Purification of Corticosteroids by HPLC-Scalability and Loadability using Agilent Prep C18 HPLC Columns", Agilent Technologies, publication 5989-2484EN, www.agilent.com/chem

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