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Application News

No. LC-23-ADI-047

UFPLC

Isolation and identification of Fluticasone degradation impurities by UFPLC

❑ Introduction

Fluticasone propionate, a medium-potency synthetic corticosteroid, is used topically to relieve inflammatory and pruritic symptoms of dermatoses and psoriasis, intranasally to manage symptoms of allergic and non-allergic rhinitis, and orally for the treatment of asthma. Fluticasone Proprionate is marketed under several different brand names such as Flonase®. Fluticasone propionate is also available as a combination product of Azelastine Hydrochloride and Fluticasone Propionate called Dymista™. Dymista™ is indicated in patients over 12 years old for symptomatic relief of seasonal allergic rhinitis.

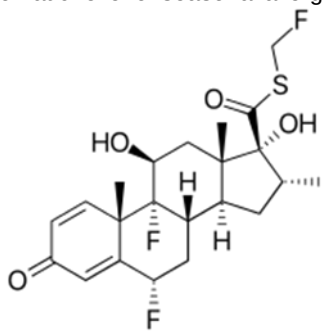


Fig. 1 Chemical structure of Fluticasone

Prominence UFPLC, Ultra Fast Preparative and Purification Liquid Chromatograph (Shown in Figure 2.), which enables fast recovery of highly purified target compounds from complex samples such as organic synthesis reaction mixtures and natural products. Prep LC is a widely used technique in many research development and manufacturing applications, including the synthesis of new drug compounds, the discovery of active components in natural products, and as a mechanism to collect large amounts of unknown compounds in foods and drugs for subsequent structural analysis.

Figure 2. UFPLC

❑ Features

- ✓ Comprehensive Automation of Preparative LC, Concentration, Purification, Elution, Collection and powderization only in 1.5 hours
- ✓ High purity as a Free Base
- ✓ Small footprint and Low-initial-cost

❑ Experimental

Base Degradation

1.0 g of Fluticasone API sample was dissolved in 25mL of methanol and added 25mL of 5% NaOH and kept at 50^{0}C for 24 hr. After the degradation evaporated methanol. This solution was neutralized with conc. HCl. Filtered the solution to recover the solid mass of degradation compound. Prepared 20000 ppm of degradation compound in water : MeOH (50:50) used it for analysis on UFPLC for fraction collection. The UFPLC parameters are shown in table No.1 Taken 10µL and diluted with 1mL of MeOH : water (1:1) to make 200 ppm and then injected in HPLC and MS to monitor fraction collection.

Table 1 Analytical Conditions

❑ Analytical Conditions

System	: Ultra fast Purification Liquid chromatography (UFPLC)
Column	: ShimPak C-18 (250X10mm, 5µ)
Mobile phase A	: 0.1% Trifluoroacetic acid in water
Mobile phase B	: Acetonitrile
Gradient program	: (0.01/ 45, 13.00/70, 17.00/80, 17.50/45, 25.00/45 time in mins/% B Conc.)
Flow Rate	: 7.0 ml/min
Wavelength	: 240 nm

❑ Results and Discussion :

Automation of Preparative LC, Concentration, Purification, Elution, Collection controlled by dedicated automation software assists chemists in clearly identifying the peaks which are trapped and collected in specific color code. 1D chromatogram is shown in Figure 3 and corresponding area percentages are given in Table 2

Table 2:Area Percentage

Peak#	Name	Ret. Time	Area%
1	Unknown peak 1	9.357	31.20
2	Unknown peak 1	12.786	39.90
3	Unknown peak 3	13.119	19.05
4	Fluticasone	14.150	9.84

The UFPLC system is capable of trapping maximum 5 peaks in one injection run on 5 different trap columns. It also rinses the individual trap columns by different rinsing solution to remove salts. It ensures that the compound is in the form of free base before it elutes. High retention capacity of trap columns can retain compounds of different polarity.

Additionally, rinsing the column with an aqueous ammonia solution after trapping allows compounds to be recovered as free bases, which are generally easier to powederize and typically yields greater quality result when used in drug screening and pharmacokinetic studies.

Fluticasone degradation solution was injected on UFPLC to collect different impurity peaks as shown in Figure 3. The fractions were collected as free base after online rinsing and desalting.

The collected fractions of individual peaks were injected on Nexera UHPLC system to check the purity. The individual chromatograms are shown in Figure 4,5,6 & 7. The degradation solution was also injected on LCMS/MS to check the m/z of degradation impurities. The collected purified fractions were also injected on LCMS/MS to confirm the m/z of the impurities.

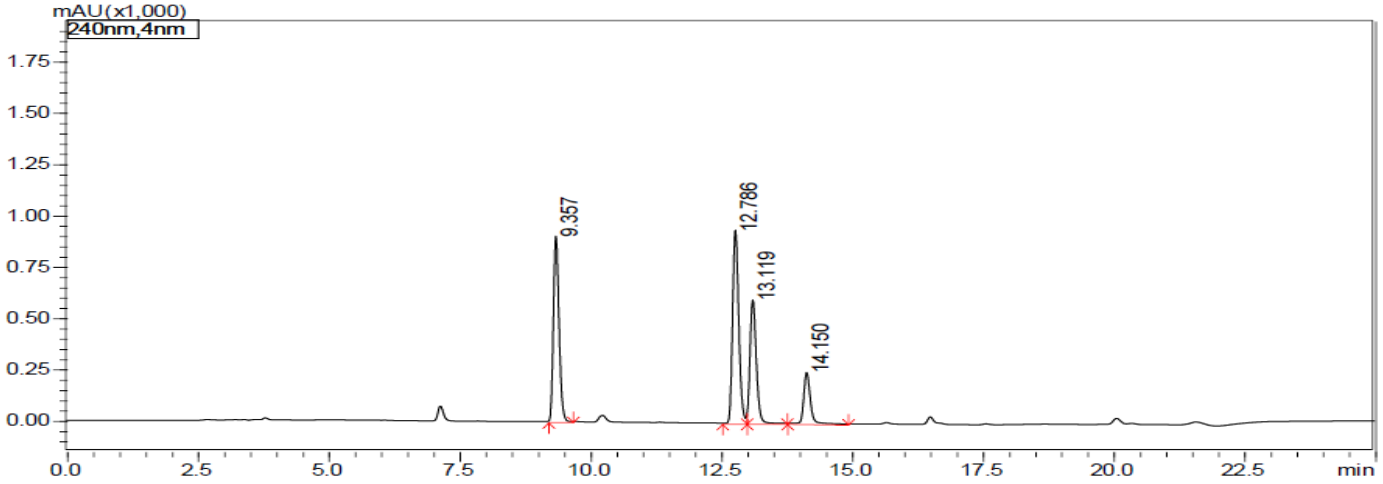


Figure 3. Crude

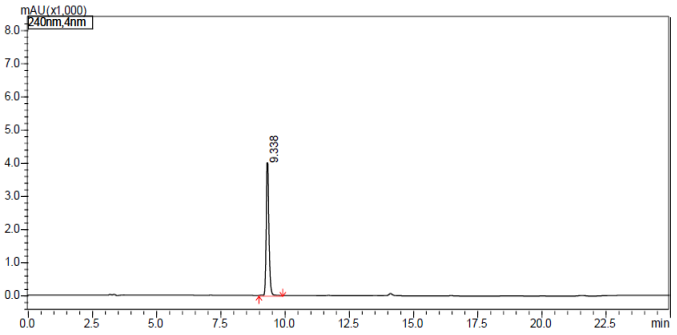


Figure 4. Peak 1

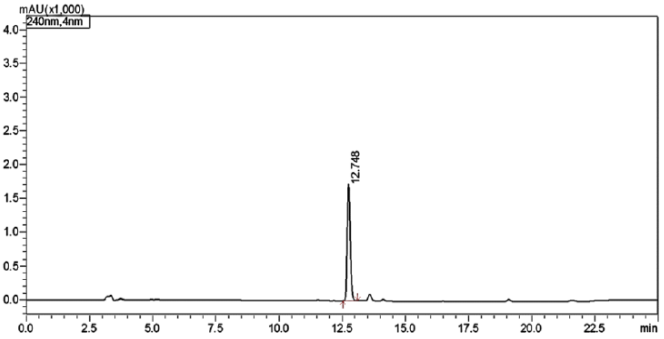


Figure 5. Peak 2

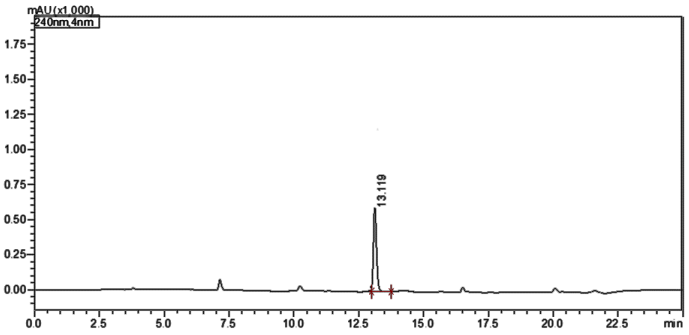


Figure 6. Peak 3

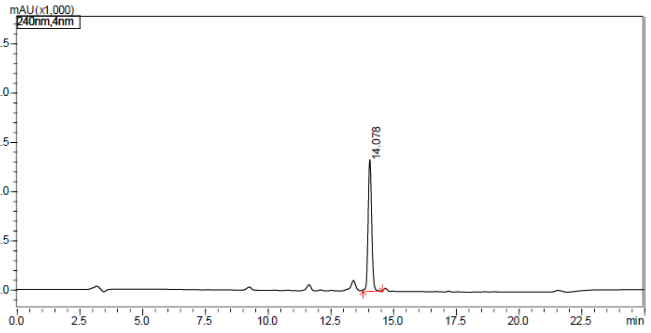


Figure 7. Peak 4

The individual chromatograms of the impurities injected after fraction collection to verify the mass of the collected impurities are shown in Figure 8, 9,10 & 11..

Fluticasone degradation impurities showed m/z of 274.00, 540.25, 469.20 and 501.20. The 4th peak of m/z 501.20 is due to Fluticasone.

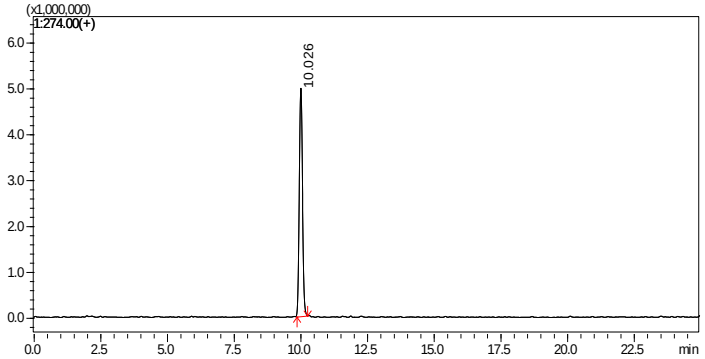


Figure 8. TIC of peak 1

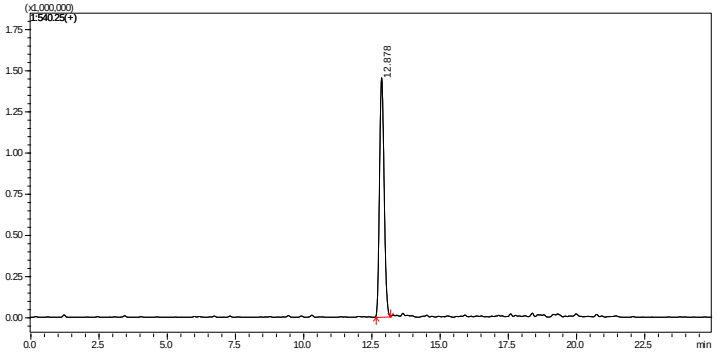


Figure 9. TIC of peak 2

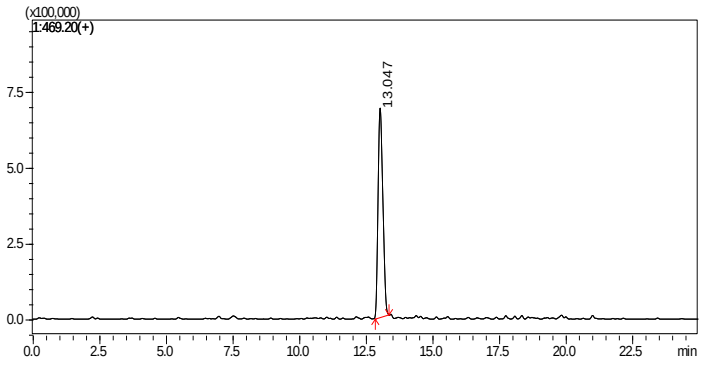


Figure 10. TIC of peak 3

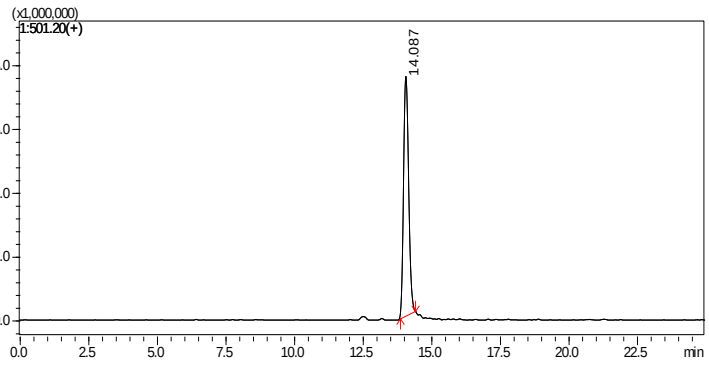
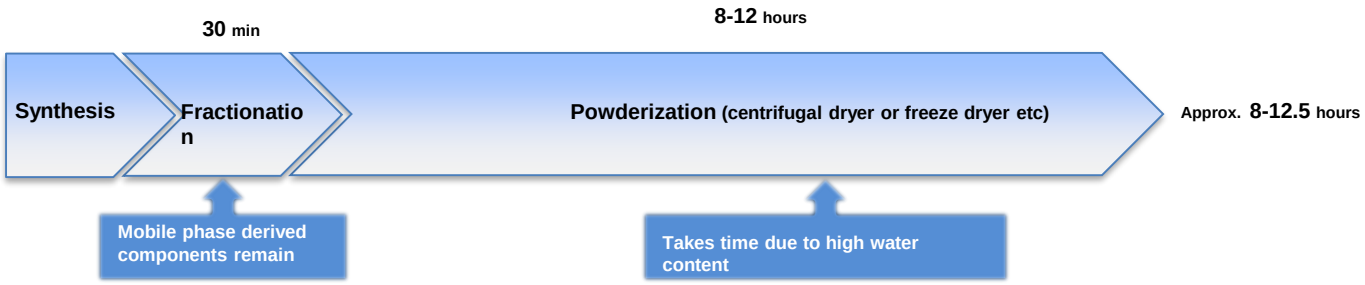


Figure 11.TIC of peak 4 Fluticasone

The Prominence UFPLC seamlessly integrates traditional Prep LC with novel fraction trapping for up to five compounds of interest. The instrument is controlled by a dedicated walk-up software designed to empower non-expert users to easily set conditions for chromatographic separation and isolation of target compounds, trapping, purifying, eluting and collecting highly purified compounds in as little as 90 minutes.(shown figure 12) For applications involving the isolation of low concentration targets, replicate injection and collection to the same trapping column to increase the amount of compound trapped on column prior to elution is easily accomplished.

The Prominence UFPLC eliminates some of the problems associated with conventional Prep LC, especially poor purity of collected compounds due to mobile phase additives, which become contaminants in the final collected fraction and inhibit powderization. Shimadzu's "Shim-pack C2P-H" trapping column strongly retains target compounds allowing unwanted organic solvents, water and additives to be flushed away. Additionally, rinsing the column with an aqueous ammonia solution after trapping allows compounds to be recovered as free bases.
With the dedicated Purification Solution software, the analysis status can be quickly confirmed at a glance using the peak tracking function.(shown in figure 13)

>>Existing method



>>Prominence UFPLC

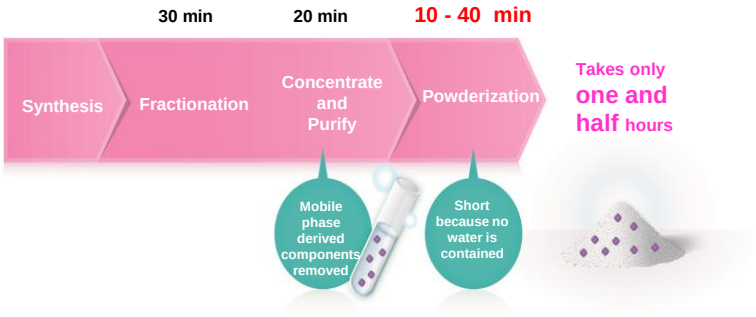


Figure 12. UFPLC purification cycle

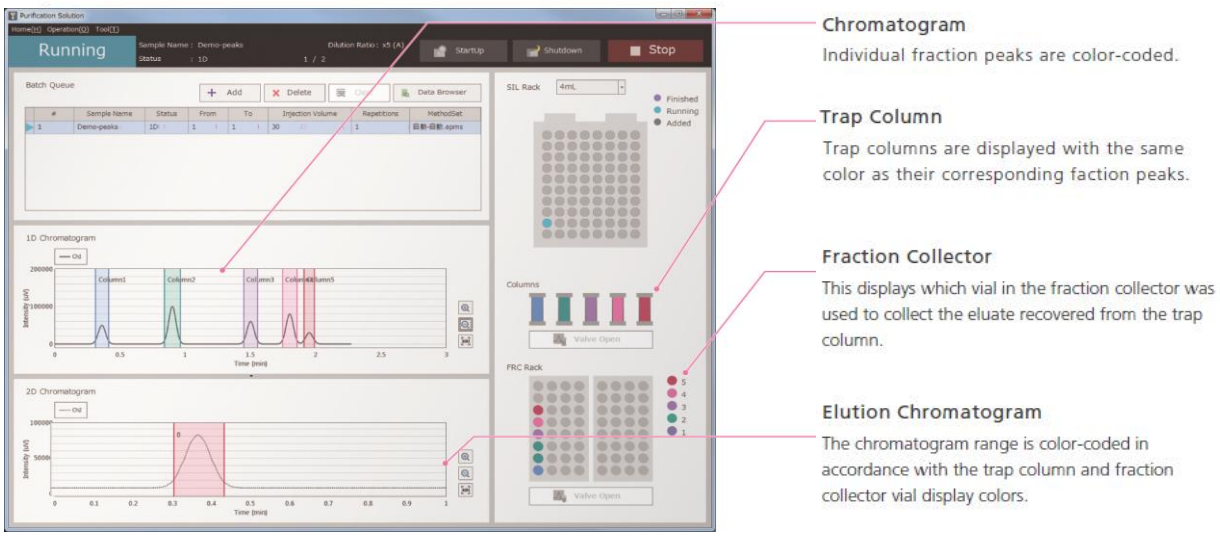
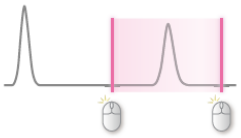
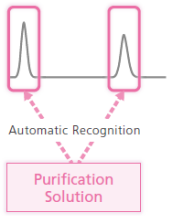


Figure 13. Purification Solution software

To ensure reliable fractionation and purification of precious samples, the Purification Solution software offers three fractionation modes



Manual Fractionation Mode
In this mode, the mouse pointer is used to fractionate peaks while viewing the window. When the same sample is concentrated by repeated injections, the first fractionation range is saved and the second and subsequent samples are automatically fractionated using the same fractionation range.



Automatic Fractionation Mode
In this mode, the software automatically identifies peaks and collects fractions based on parameter settings.

Time-Specified Fractionation Mode
This mode collects fractions based on the retention times in previously acquired data. It is ideal for routinely performed preparative purification processes.

❑ Conclusion

The Prominence UFPLC seamlessly integrates traditional Prep LC with novel fraction trapping for upto five compounds of interest. The instrument is controlled by a dedicated walk-up software designed to empower non-expert users to easily set conditions for chromatographic separation and isolation of target compounds, trapping, purifying, eluting and collecting highly purified compounds in as little as 90mins. For applications involving the isolation of low concentration targets, replicate injection and collection to the same trapping column to increase the amount of compound trapped on column prior to elution is easily accomplished.

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