SHIMADZU

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Unknown Constituent Identification in Topical Preparation using a Q-TOF Mass Spectrometer

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1. Overview

Using a high-resolution mass spectrometer and a new qualitative analysis software (Assign) to identify low level unknowns in topical preparations.

2. Introduction

Skin is the most exposed organ in the human body and is susceptible to various diseases and disorders. Topical preparations are often used to provide treatment for skin since it is easily administered. Although most topical preparations are meant to provide treatment locally, a small amount of those drugs, including the active compounds and other constituents, can be absorbed and lead to systemic effect. To avoid undesirable effects caused by the unknown constituents in the topical preparation, an LCMS method utilizing a Shimadzu LCMS-9030 Q-TOF was created to analyze topical preparation products. A typical workflow on data analysis was developed for conducting unknown constituent analysis in topical preparation.

3. Method

A topical preparation was commercially obtained and evaluated for unknown constituent analysis. Qualitative analysis of the unknown constituent was performed on a Shimadzu LCMS-9030 Q-TOF mass spectrometer. Both the LC and MS parameters are outlined in Table 1. This poster will explore the typical workflow for conducting qualitative analysis of an unknown constituent. (Figure 1)

Table 1 LC-MS conditions and parameters

LC -30AD		LCMS-9030	
Column:	Shimadzu ODS-III column (150 x 2.1 mm)	**Nebulizing Gas:**	2 L/min
Mobile phase:	A: 0.1 % Formic acid in water; B: 0.1 % Formic acid in Acetonitrile	**Heating Gas:**	10 L/min
Flow Rate:	0.4 mL/min	**Interface Temp.:**	300 °C
Oven Temp.:	40°C	**DL Temp.:**	250 °C
Injection Vol.:	1 µL	**Heat Block Temp.:**	400 °C
UV wavelength:	237 nm	**Drying Gas:**	10 L/min
		Scan range (m/z):	100-1000

Qualitative Analysis Workflow

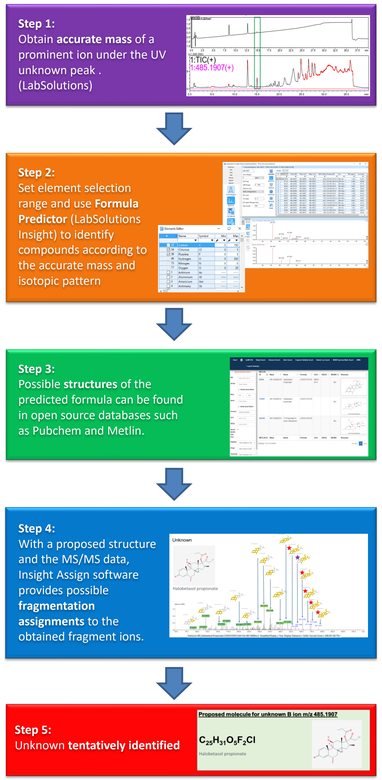


Figure 1 A typical workflow for analysing an unknown constituent using a Q-TOF mass spectrometer.

4. Results

4-1. LC-MS

To mimic a standard pharmaceutical unknown analysis, tandem UV and MS data were acquired. The unknown preparation contains an additional peak (Rt=15 min) that does not exist in the control preparation. Accurate masses and the MS/MS fragments of the main ingredient, diflorasone diacetate (Rt=13 min; m/z 495.2186) and the prominent ion (Rt=15 min; m/z 485.1907) under the unknown peak were acquired. (Figure 2)

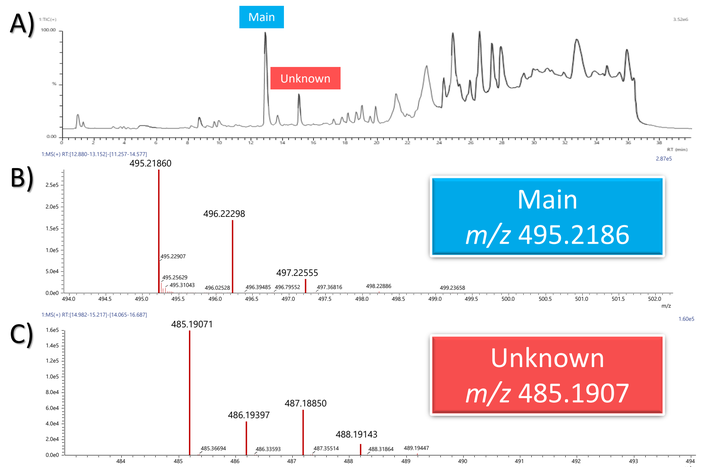


Figure 2 A) TIC of the unknown sample showing both the main and the unknown peaks. B) Mass spectrum of the main ingredient, diflorasone diacetate, with m/z 495.2186, and C) mass spectrum of the unknown ion, m/z 485.1907. Distinct isotopic distributions and ratio were observed.

4-2. LC-MS/MS

MS/MS fragment patterns and assignments results exhibit the similarity between the main ingredient and the unknown compound (similar fragments highlighted in green). The results also suggest the differences between the unknown constituent and the main ingredient are expected to be on the two functional groups circled in red and purple on the diflorasone diacetate structure.

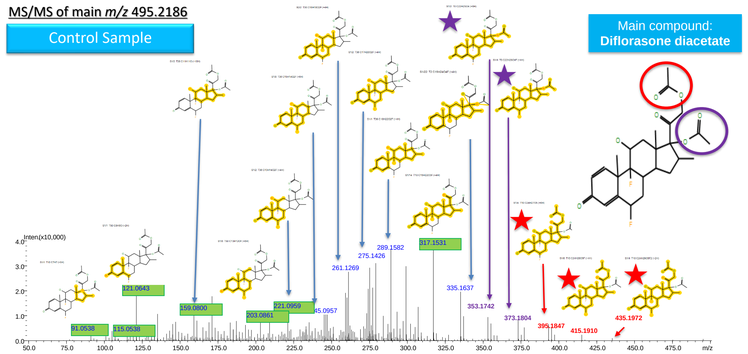


Figure 3 Fragment structure assignment results from the Shimadzu Insight Assign software. MS/MS data obtained by the Q-TOF on the main ingredient, ion m/z 495.2186, and the known structure of diflorasone diacetate were used for the prediction.

4-3. Formula prediction and fragment assignment results of the unknown constituent

Based on the clues provided by the accurate mass, isotopic distribution, and MS/MS fragmentation patterns of the unknown and main ingredient, a proper range of possible elements for formula prediction was set. Sixteen potential formulas were predicted for the unknown ion. (Table 2) The best matched formula according to accurate mass, isotopic distribution, and isotopic ratio was $C_{25}H_{31}O_5F_2Cl$. Two possible structures, Halobetasol Propionate and 11-Propionate 21-chlorodiflorasone, were matched to the predicted formula of the unknown in an open source database (Metlin). Shimadzu Insight Assign software was then used to assign the MS/MS fragments of the unknown against the possible fragments of the two possible structures. (Figure 4) Halobetasol Propionate had a closer match (82.7%) in fragment assignment of the unknown's fragmentation spectrum. Therefore, Halobetasol Propionate was tentatively identified as the unknown compound.

Table 2 Formula prediction results of the unknown ion (m/z 485.1907).

#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
1	**96.98**	**484.18281**	**485.19008**	**485.19072**	**0.64**	**C25 H31 O5 F2 Cl**	**[M+H]+**	**1.310**	**97.35**	**9.0**
2	91.14	484.18395	485.19123	485.19072	-0.51	C22 H32 O6 F3 Cl	[M+H]+	-1.046	90.69	5.0
3	88.45	462.20205	485.19127	485.19072	-0.55	C22 H35 O8 Cl	[M+Na]+	-1.126	87.75	5.0
4	86.80	462.20179	485.19101	485.19072	-0.29	C26 H29 O4 F3	[M+Na]+	-0.607	96.72	11.0
5	62.00	484.18167	485.18894	485.19072	1.78	C28 H30 O4 F Cl	[M+H]+	3.665	60.88	13.0
6	57.52	462.20294	485.19216	485.19072	-1.44	C23 H30 O5 F4	[M+Na]+	-2.963	66.03	7.0
7	56.01	462.20065	485.18987	485.19072	0.85	C29 H28 O3 F2	[M+Na]+	1.748	63.24	15.0
8	54.22	484.18256	485.18983	485.19072	0.89	C29 H25 O F5	[M+H]+	1.829	61.31	15.0
9	48.90	462.19960	485.18882	485.19072	1.90	C20 H34 O6 F3 Cl	[M+Na]+	3.913	46.80	2.0
10	46.97	484.18386	485.19113	485.19072	-0.41	C34 H25 O2 F	[M+H]+	-0.855	52.61	22.0
11	45.48	484.18509	485.19237	485.19072	-1.65	C19 H33 O7 F4 Cl	[M+H]+	-3.401	42.10	1.0
12	44.70	462.20319	485.19241	485.19072	-1.69	C19 H36 O9 F Cl	[M+Na]+	-3.482	41.35	1.0
13	42.97	484.18272	485.18999	485.19072	0.73	C37 H24 O	[M+H]+	1.501	48.57	26.0
14	29.60	484.18500	485.19228	485.19072	-1.56	C31 H26 O3 F2	[M+H]+	-3.210	35.31	18.0
15	21.77	462.19951	485.18873	485.19072	1.99	C32 H27 O2 F	[M+Na]+	4.104	28.20	19.0
16	21.49	484.18141	485.18869	485.19072	2.03	C32 H24 F4	[M+H]+	4.184	28.11	19.0

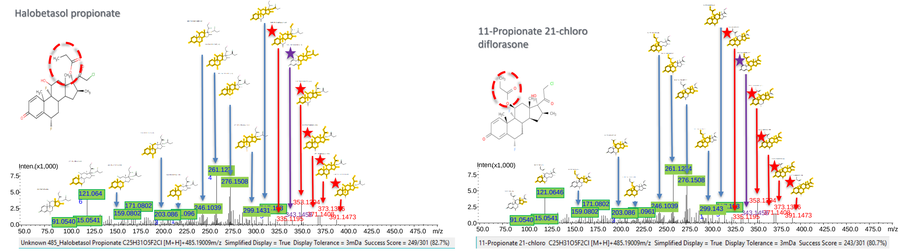
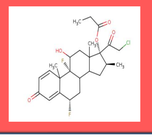


Figure 4 Fragment structure assignment results of the two possible unknown structures, Halobetasol Propionate and 11-Propionate 21-chlorodiflorasone, from the Shimadzu Insight Assign software.

Proposed molecule for unknown ion *m/z* 485.1907 (Rt = 15 min) in the unknown topical preparation sample

$C_{25}H_{31}O_5F_2Cl$

Halobetasol propionate



5. Conclusions

The Shimadzu Q-TOF mass spectrometer with the Shimadzu Formula Prediction and Assign software provide a simple and straightforward qualitative analysis workflow. In this experiment, LCMS-9030 demonstrated the excellent capabilities to identify low level unknowns in a complex extraction sample.