Qualitative Analysis of Gossypol, Free Gossypol, and Gossypol Derivatives in the Cottonseeds By Electrospray Ionization Tandem Mass Spectrometry

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Introduction

Free gossypol and in cottonseed is a toxic chemical, which can affect human reproductive function, skin and stomach burning, nausea, vomiting, diarrhea, headache, coma, convulsions and even death due to respiratory, circulatory system failure. Therefore, strict control of the free gossypol and its derivatives is required in deep-processing process of cottonseed. In this Study, according to the MS^n Spectra of gossypol, the structures of known and unknown gossypol derivatives were figure out by LCMS-IT-TOF.

Experimental

Analytical Conditions

HPLC (UHPLC system)

The analysis were performed on a Shimadzu UFLC$_{XR}$-20A system equipped with LC-20AD$_{XR}$ pump, CTO-20AX column oven, SIL-20AC$_{XR}$ auto sampler, DGU-20A$_5$ Degasser and CBM-20A controller.
Mobile phase: A –0.1%formic acid-wate, B – acetonitrile
Column   : Shimadzu Shim-pack XR-ODS II
                   (2.0 mm×75 mm, 2.2 µm)
Flow rate   : 0.2 mL/min
Column oven   : 40°C
Injection volume : 10 µL
Gradient program: showed in Table 1

Mass (LCMS-IT-TOF)

Interface: ESI -, Interface voltage: - 4.5 kV, Scan Range: *m/z* 100-1100, Nebulizing gas: N2, 1.5 L/min, Drying gas: N2, 10 L/min, Collision gas: Ar^2, CDL temperatue: 200°C, Heat block temperatue: 250°C, Detector voltage: 1.70 kV
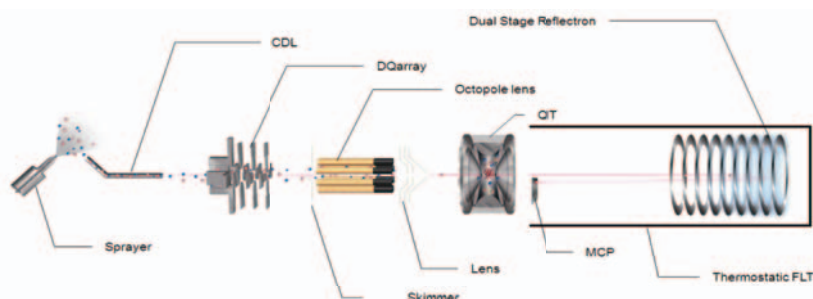
Table 1 LC Gradient Program

Time (min)	B.Conc %
0.01	30
5.00	70
10.00	85
13.00	85
15.00	95
18.00	95
18.01	30
25.00	30

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Results and Discussion

Gossypol Standard Analysis

2 μg / mL of gossypol standard samples analyzed by LCMS-IT-TOF. The retention time of gossypol was 11 min. The chromatogram of gossypol was shown in Fig. 2.
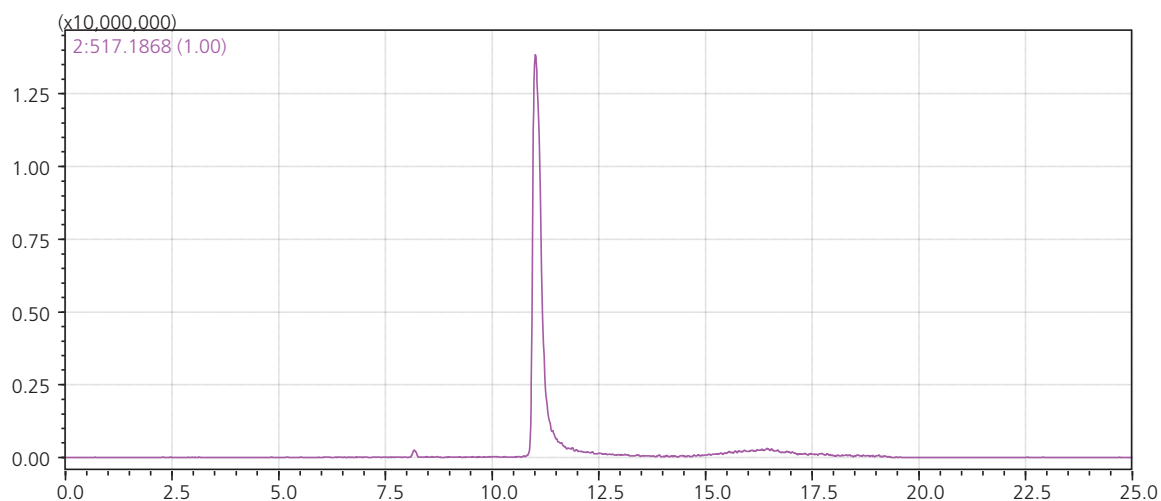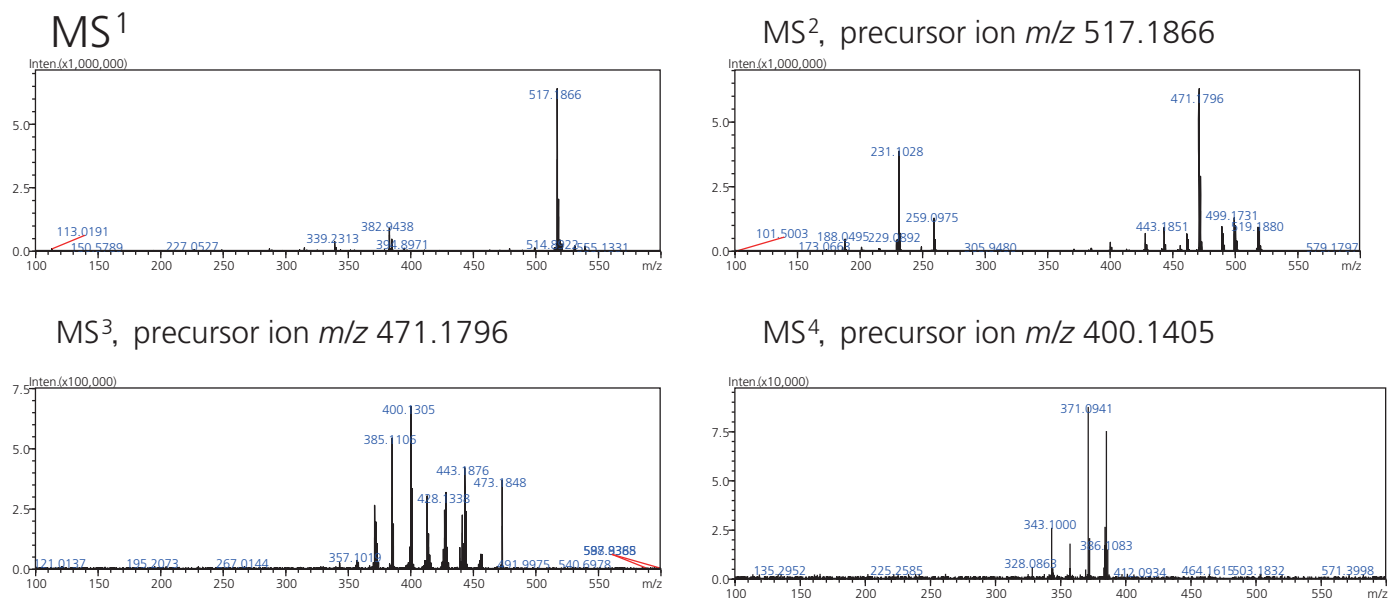
Fig. 2. Chromatogram of gossypol

MS^1

MS^2, precursor ion m/z 517.1866

MS^3, precursor ion m/z 471.1796

MS^4, precursor ion m/z 400.1405

Fig. 3. MS^n Spectrum of Gossypol

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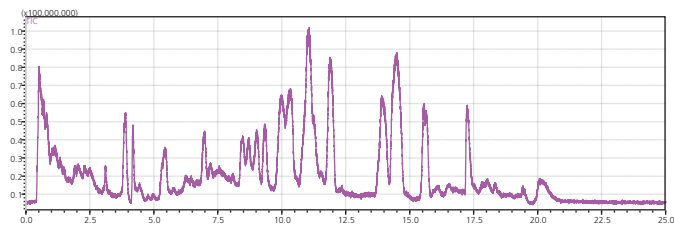## Analysis of Free Gossypol and Its Derivatives in Cottonseed

Fig. 5 Chromatogram of Free Gossypol and Its
Derivatives in Cttonseed Extracted by
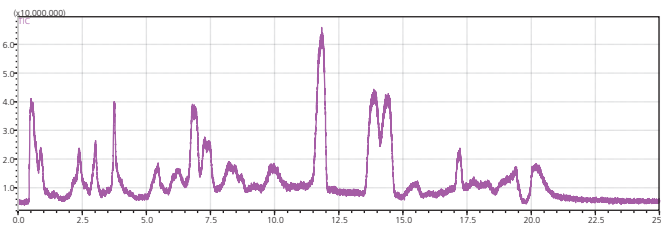Chloroform / ethanol / isopropyl alcohol (40/40/20)

Fig. 6 Chromatogram of Free Gossypol and Its
Derivatives in Cttonseed Extracted by
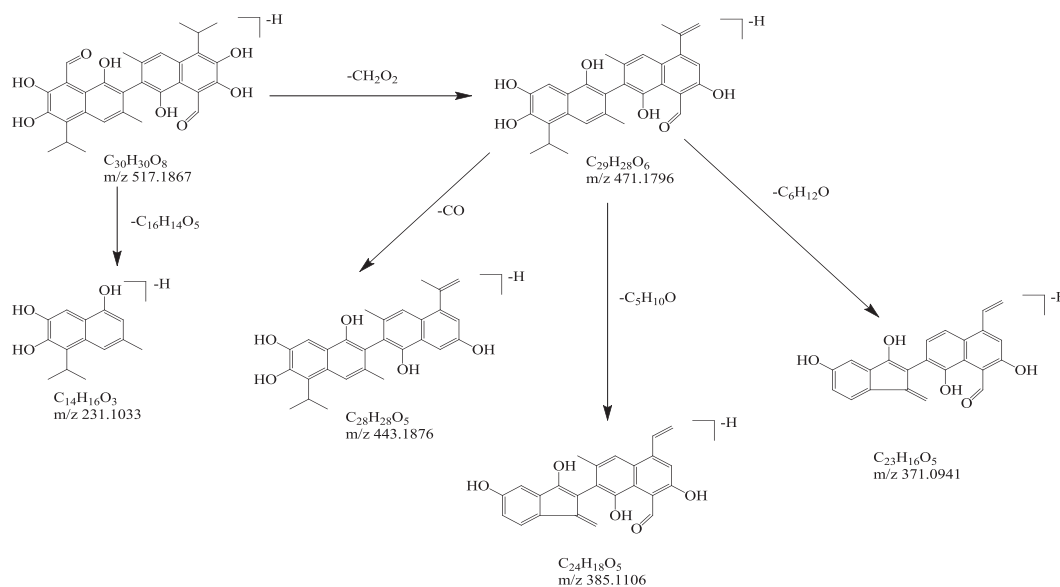Acetonitrile / 0.2% phosphoric acid (85/15)

Fig. 4 Mass Fragmentation of Gossypol

Table 2 Free Gossypol and Its Derivatives in Cttonseed Extracted by Chloroform / ethanol / isopropyl alcohol

No.	Retention Time (min)	Formula	Ion Type	Theoretical *m/z*	Measure *m/z*	Mass Difference (ppm)
1	2.4	$C_{15}H_{10}O_7$	$[M-H]^-$	301.0354	301.0368	4.65
2	3.0	$C_{15}H_{10}O_6$	$[M-H]^-$	285.0405	285.0414	3.16
3	3.8	$C_{18}H_{34}O_4$	$[M-H]^-$	313.2384	313.2390	1.92
4	5.4	$C_{18}H_{34}O_4$	$[M-H]^-$	313.2384	313.2394	3.19
5	6.2	$C_{18}H_{36}O_4$	$[M-H]^-$	315.2541	315.2554	4.12
6	6.9	$C_{18}H_{32}O_3$	$[M-H]^-$	295.2279	295.2286	2.37
7	7.4	$C_{18}H_{34}O_3$	$[M-H]^-$	297.2435	297.2448	4.37
8	11.8	$C_{16}H_{28}O_{10}$	$[M-H]^-$	379.1610	379.1623	3.43
9	14.4	$C_{29}H_{28}O_8$	$[M-H]^-$	381.1766	381.1757	-2.36

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Table 3 Free Gossypol and Its Derivatives in Cttonseed Extracted by Acetonitrile / 0.2% phosphoric acid

No.	Retention Time (min)	Formula	Ion Type	Theoretical *m/z*	Measured *m/z*	Mass Difference (ppm)
1	5.5	$C_{18}H_{34}O_4$	$[M-H]^-$	313.2384	313.2394	3.19
2	5.6	$C_{18}H_{34}O_4$	$[M-H]^-$	313.2384	313.2400	5.11
3	6.3	$C_{18}H_{36}O_4$	$[M-H]^-$	315.2541	315.2547	1.90
4	6.5	$C_{34}H_{47}NO_6$	$[M-H]^-$	564.3331	564.3347	2.84
5	7.0	$C_{18}H_{22}O_3$	$[M-H]^-$	295.2279	295.2281	0.68
6	7.5	$C_{18}H_{24}O_3$	$[M-H]^-$	297.2435	297.2442	2.35
7	7.7	$C_{24}H_{28}O$	$[M-H]^-$	331.2067	331.2053	-4.2
8	8.9	$C_{19}H_{30}N_2O_3$	$[M-H]^-$	333.2184	333.2189	1.50
9	11.1	$C_{30}H_{30}O_8$	$[M-H]^-$	517.1868	517.1852	-3.09
10	11.9	$C_{16}H_{28}O_{10}$	$[M-H]^-$	379.1610	379.1602	-2.11
11	14.0	$C_{19}H_{20}N_2O_5$	$[M-H]^-$	355.1299	355.1291	-2.25
12	14.6	$C_{16}H_{30}O_{10}$	$[M-H]^-$	381.1766	381.1766	-1.57

Conclusions

According to the MS^n spectrums of gossypol and its derivatives, quantity and quality of free gossypol derivatives were different when using different extracting way. The qualitative results of gossypol derivatives get from LCMS-IT-TOF could tell the best way of deep-processing process of cottonseed.

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