PO-CON1741E

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An Ultrafast LC/MS/MS Method for Characterization and Quantitation of Triton X-100 Extracted From Palm Oil

ASMS 2017 TP 712

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Introduction

Triton X-100, oligomers of octylphenol ethoxylate (OPEOn), is predominantly used as detergent in laboratory, industry and household [1]. The chain lengths of ethoxy units (n) of the oligomers ranged from 0 to 20 (Figure 1). Analysis of octylphenol ethoxylates by GC, HPLC and LC/MS methods were reported previously for monitoring the residues in river and wastewater due to their potential toxicity towards aquatic ecosystem [2-4]. In the last decade, oil-bearing crops has gained much attention for use as a raw material for biofuel. Palm oil (*Elais guineensis*) is one of the commercialized and most profitable oil-bearing crops to date. It has been widely used as a traditional cooking oil in Southeast Asia and Africa and commonly found in processed food, makeup, toothpaste and cleaning products. There is inquiry recently if Triton X-100 is present in edible palm oil and food products due to its involvement in the extraction process for palm oil production. We describe here an ultrafast LC/MS/MS method for sensitive analysis of Triton X-100 in palm oil samples.

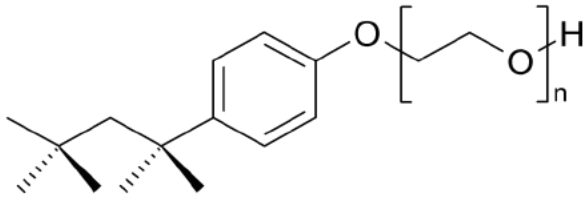

Figure 1. Chemical structure of the octylphenol ethoxylates (OPEOn) investigated in this study.

Experimental

Triton X-100 standard was acquired from Sigma Aldrich. An edible palm oil was obtained from local supermarket and utilized as matrix for method development. A customized liquid-liquid extraction (LLE) using acetonitrile-saturated hexane (1:1, v/v) was employed for the extraction of Triton X-100. The sample pretreatment procedure and LC/MS/MS method are described in Figure 2 and Table 1, respectively.

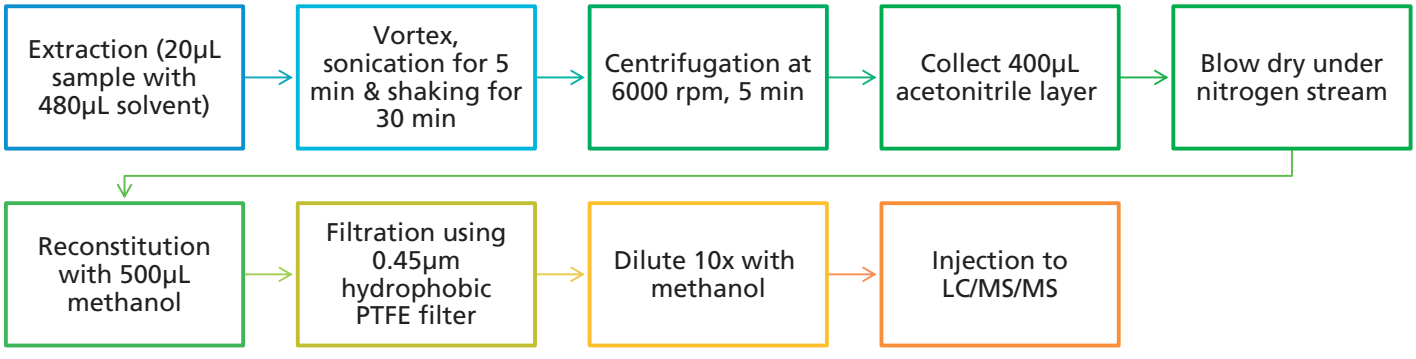

Figure 2. Flow chart of sample pretreatment.

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Table 1. Analytical conditions of Triton X-100 on LCMS-8060.

Column	: Kinetex C18 100Å (50 x 2.1 mm, 1.7 µm)
Flow rate	: 0.3 mL/min
Mobile phase	: A: Water with 2mM ammonium acetate B: Acetonitrile with 2mM ammonium acetate
Elution mode	: Gradient elution, 0min (50% B) → 0.01-2.5mins (100% B) → 2.51-3.00mins (100% B) → 3.01-3.50mins (50% B) → 3.51-5.00 mins (50% B)
Oven temp.	: 30 °C
Injection vol.	: 5.0 µL
Interface & temp.	: ESI, 300 °C
MS mode	: Positive, MRM
Block temp.	: 400 °C
DL temp.	: 250 °C
CID gas	: Ar (270 kPa)
Nebulizing gas flow	: N_2, 2 L/min
Drying gas flow	: N_2, 15 L/min
Heating gas flow	: 0 air, 10 L/min

Results and Discussion

Development of LC/MS/MS method

A MRM-based method for quantitation of Triton X-100 was developed. The OPEOn species forms ammonia adduct ion $[M+NH_4]^+$ under the mobile phase and ESI conditions. Using an automated MRM optimization program on LCMS-8060, each OPEOn adduct ion was subjected to optimize for highest intensity for every MRM transition. It was observed that the most intensive MRM transition of OPEOn species (n = 3~20) is not the transition losing ammonia. Two MRM transitions were selected for each OPEOn species, with the higher intensity one as the quantifier (Table 2) and the other for confirmation. A total of eighteen species of OPEOn with n = 3~20 were analysed. A characteristic pattern of peak distribution with OPEO8 peak highest is observed. The RT of the species decreases with the number of ethoxy units (*n*). The total MRM chromatograms (sum of two MRMs for each species) of the 18 OPEOn are displayed in Figure 3.

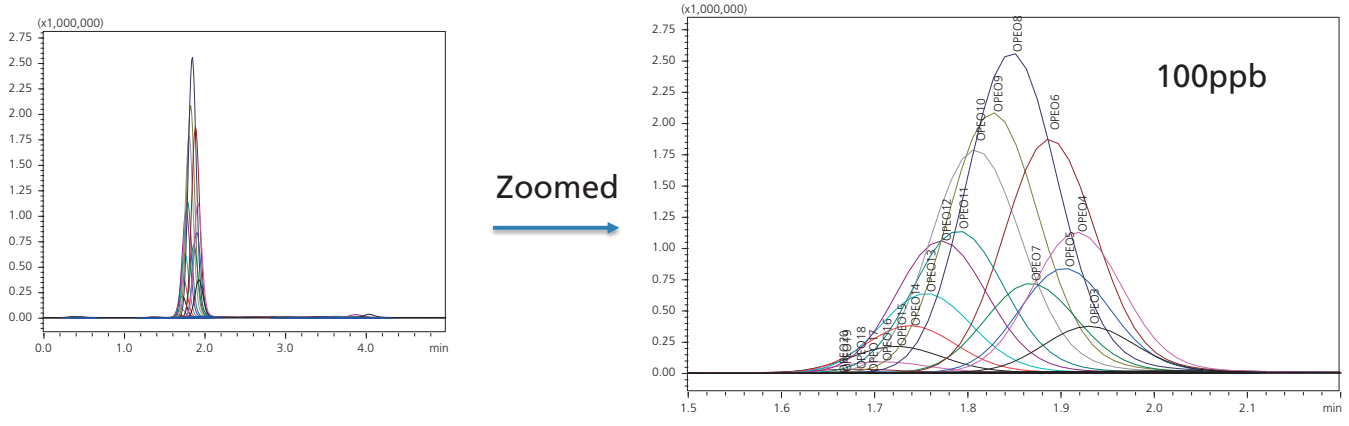

Figure 3. Total MRM chromatograms (each is the sum of two individual MRMs of the OPEOn) of 18 species of Triton X-100 standard (n = 3~20) at 100 ppb.

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Quantitation performance with edible palm oil spiked sample

The MRM-based calibration curves (Figure 4) was established with palm oil spiked samples. Effective extraction of the OPEOn from palm oil matrix was found challenging in the study. Various extraction procedures including LLE, solid phase extraction (SPE) and dispersive SPE (dSPE) were applied and compared to extract Triton X-100 from palm oil. The extraction efficiency was determined with spiked samples. Triton X-100 standard dissolved in methanol was spiked into palm oil at low, mid, and high concentrations (8, 40 and 80 ppb). A LLE with acetonitrile-saturated with hexane (1:1, v/v) produced the best recovery ranged from 74.4 to 127.8% and without severe matrix effect (75.5 to 136.6%).

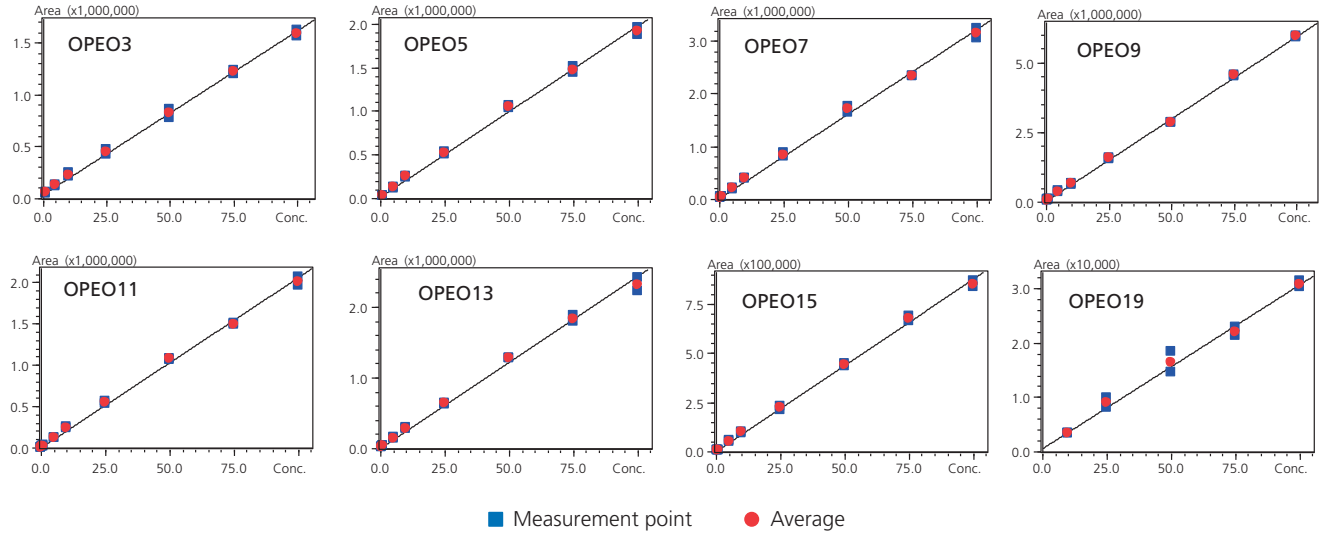

Figure 4. Representative calibration curves of OPEO species in oil matrix (two replications, *n*=2).

The calibration curves were built using palm oil matrix spiked with Triton X-100 standard. Linear relationship with $R^2 \geq 0.994$ were obtained for all OPEO species across minimum five quantitation points from 0.5 or 1 ppb to 100 ppb for most OPEO species except for the species of n = 17, 19 and 20 (Table 2).

Most of the OPEO species exhibited a detection limit (LOD) based upon S/N > 3 in the range of 0.02~0.8 ng/mL level and a quantitation limit (LOQ) method based upon S/N > 10 of approximately lower than 3 ng/mL, except for OPEO17, OPEO19, and OPEO20. The reduced sensitivity for OPEO with a higher number of ethoxy units is probably because of poorer ionization and fragmentation efficiency of the molecules. The repeatability of the method was evaluated at 50 ppb for all OPEO species. The RSD of most species were under 8% across six repetitive injections, except for three OPEO species (OPEO17, 19, &20). The results are summarized in Table 2.

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Table 2. Calibration results of Triton X-100 standard in edible palm oil matrix (two replications, *n*=2).

No	Triton X-100 species	MRM transition for quantitation	RT (min)	LOQ (ppb)	LOD (ppb)	Range (ppb)	Linearity (R^2)	Repeatability at 50 ppb*	
								Area RSD (%)	Conc. RSD (%)
1	OPEO3	365.25>227.15	1.93	0.21	0.07	1-100	0.998	6.09	6.35
2	OPEO4	400.25>271.10	1.92	1.25	0.41	1-100	0.999	3.97	4.04
3	OPEO5	444.25>133.05	1.91	1.29	0.43	1-100	0.997	3.63	3.68
4	OPEO6	488.25>471.25	1.89	0.65	0.22	1-100	0.997	1.79	1.81
5	OPEO7	532.35>277.20	1.87	0.81	0.53	0.5-100	0.997	4.75	4.79
6	OPEO8	576.35>559.35	1.85	0.85	0.28	0.5-100	0.998	2.93	2.95
7	OPEO9	620.40>603.40	1.83	0.59	0.2	0.5-100	0.998	4.66	4.7
8	OPEO10	664.40>133.10	1.81	0.07	0.035	0.5-100	0.998	5.52	5.55
9	OPEO11	708.40>177.10	1.79	0.06	0.02	0.1-100	0.997	3.07	3.08
10	OPEO12	752.45>133.05	1.78	0.09	0.03	0.5-100	0.997	3.72	3.75
11	OPEO13	796.50>133.00	1.76	0.67	0.22	0.5-100	0.997	4.3	4.3
12	OPEO14	840.50>133.10	1.74	0.64	0.21	0.5-100	0.997	3.38	3.4
13	OPEO15	884.55>133.05	1.75	2.17	0.72	0.5-100	0.998	4.39	4.4
14	OPEO16	928.60>88.95	1.71	1.15	0.38	0.5-100	0.995	6.43	6.43
15	OPEO17	972.60>146.95	1.70	24.9	8.21	5-100	0.997	8.19	8.2
16	OPEO18	1016.70>89.00	1.68	2.3	0.76	1-100	0.997	4.58	4.6
17	OPEO19	1060.80>133.25	1.67	15.9	5.25	10-100	0.994	17.4	17.9
18	OPEO20	1104.80>133.20	1.66	69.9	23.1	50-250	0.999	11.16	11.46

*6 replications, n=6

LC/MS/MS analysis of crude palm oil

The above method was applied to a crude palm oil sample obtained from a research lab to detect the presence of Triton X-100 residues, which might be used in the extraction process of production. The matrix of crude palm oil is more complex compared to that of edible palm oil sold in supermarket. Thus, in addition to the blank crude palm oil sample, spiked samples with Triton X-100 at two concentrations (5 ppb and 50 ppb) were also analyzed to compare the baselines, interference and LC/MS/MS analysis results. The same sample extraction and purification procedure shown in Figure 2 was applied for the crude palm oil sample.

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Table 3. Results of crude palm oil analysis (blank)

No	Triton X-100 species	Crude palm oil blank sample (non spiked)*	
		Area	Conc. (ppb)
1	OPEO3	24931	ND**
2	OPEO4	22300	ND**
3	OPEO5	30932	0.68
4	OPEO6	17996	ND**
5	OPEO7	7761	ND**
6	OPEO8	14118	ND**
7	OPEO9	7176	ND**
8	OPEO10	7152	ND**
9	OPEO11	1399	ND**
10	OPEO12	2278	ND**
11	OPEO13	-	ND
12	OPEO14	-	ND
13	OPEO15	-	ND
14	OPEO16	-	ND
15	OPEO17	-	ND
16	OPEO18	-	ND
17	OPEO19	-	ND
18	OPEO20	-	ND

*three replications, *n*=3
**under detection limit (S/N < 3)

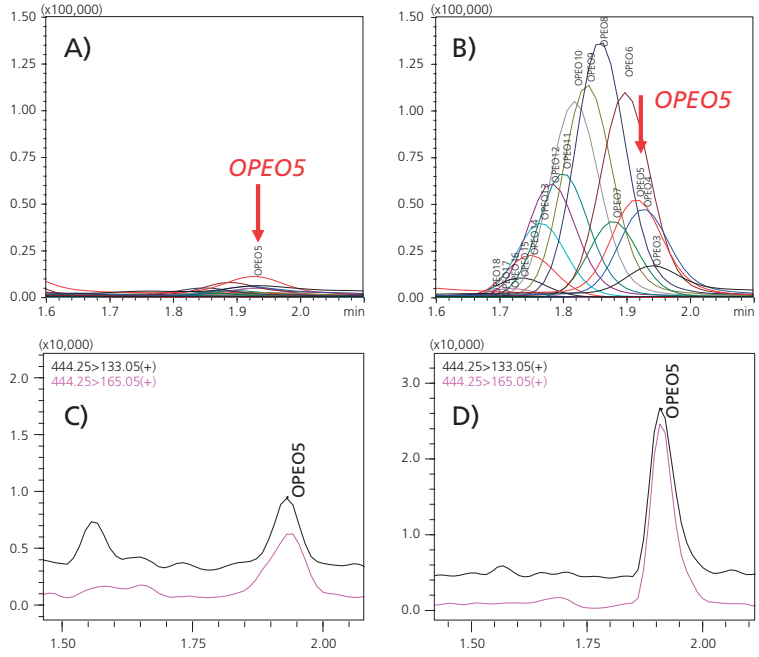

Figure 5. Total MRMs of crude palm oil (blank) (A) and spiked with 5ppb standard (B).
Individual MRMs of OPEO5 of crude palm oil (blank) (C) and spiked with 5ppb standard (D).

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The quantitative results indicate the presence of OPEO5 at very low content (~0.68 ppb) in the crude palm oil sample (Table 3 & Figure 5). However, this level is lower than the quantitation limit of the method. Despite shown with various peak area values, other 9 Triton X-100 species are considered not detected because the levels are below the detection limit (S/N < 3). The characteristic of distribution pattern of Triton X-100 species are also not observed thus the targeted Triton X-100 is unlikely presence in the sample.

Conclusions

A MRM-based method for characterization and quantitation of Triton X-100 has been established using a Kinetex C18 100Å column on LCMS-8060. A total of 18 Triton X-100 species (n = 3~20) was analysed within 5 minute running time using gradient elution program. With remarkable system sensitivity at the level of 3 ppb or less for most of Triton species, this ultrafast LC/MS/MS method is applicable to screen and quantitate the residue of Triton X-100 in crude palm oil.

References

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First Edition: June, 2017

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