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Application of time-of-flight mass spectrometry for screening of crude glycerins for toxic phorbol ester contaminants

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ABSTRACT

Since 2007, the U.S. Food and Drug Administration (FDA) has received numerous complaints of pet illnesses that may be related to the consumption of jerky pet treats. Many of those treats include glycerin as an ingredient. Glycerin can be made directly from oils such as palm seed oil, but can also be derived from the seed oil of toxic *Jatropha* plant during biodiesel production. If crude glycerin from biodiesel production from *Jatropha curcas* is used in the manufacture of animal feed, toxic tigliane diterpene phorbol esters (PEs), namely Jatropha factors (JFs), may be present and could lead to animal illnesses. Considering the numerous uses of glycerin in consumer products there is a need for a rapid method to screen crude glycerin for JF toxins and other PE contaminants. We describe the development of an ultra-high pressure liquid chromatography/quadrupole time of flight (UHPLC/Q-TOF) method for screening crude glycerin for PEs. An exact mass database, developed in-house, of previously identified PEs from *Jatropha curcas* as well as putative compounds was used to identify possible contaminants.

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1. Introduction

The Center for Veterinary Medicine at FDA has received numerous consumer reports of canine illnesses following consumption of jerky pet treats (JPT) since 2007 [1]. The majority of cases (approximately 57%) involve gastrointestinal symptoms. Renal dysfunction or failure accounts for 28% of the cases, with a smaller subset reporting Fanconi syndrome. Fanconi syndrome is a dysfunction of the proximal renal tubules of the kidney in which glucose, amino acids, uric acid, phosphate, and bicarbonate are passed into the urine instead of being reabsorbed.

Jerky pet treats (JPTs) are generally made by mixing poultry (chicken, duck, turkey, etc.) breast meat with glycerin before they are dried in an oven. Glycerin is also used extensively in the production of drugs, cosmetics, foods, tobacco products, sweeteners, toiletries, etc. Recently, the manufacture of biodiesel has generated significant amounts of crude glycerin as a by-product. Crude glycerin can have many impurities including methanol, but its low cost creates an economic incentive to illegally substitute it for food-grade glycerin in the production of JPTs. The plant *Jatropha curcas* has been extensively studied. Its seeds are composed of 30% oil and may be used for biodiesel production, especially in developing countries. Six tigliane diterpene phorbol esters (PEs), namely Jatropha factors (Fig. 1; JFs C1–C6), are present in the oil of toxic *J. curcas* varieties. JFs have been shown to have toxic properties [2–6]. There are concerns that *Jatropha curcas* derived crude glycerin may be used in food production, which could introduce toxic PEs into the food supply. In 2012, the Food and Drug Administration (FDA) issued a notice to industry at http://www.fda.gov/ForIndustry/IndustryNoticesandGuidanceDocuments/ucm391133.htm regarding oils, glycerin, and proteins commonly used in the production of FDA-regulated products, due to their potential for contamination with these toxins if the ingredients are derived from the *Jatropha* plant. Although FDA subsequently reported at http://www.fda.gov/ForIndustry/IndustryNoticesandGuidanceDocuments/ucm391140.htm that the amount of *Jatropha* used in biodiesel production in Malaysia and Indonesia may not be as marked as previously thought, the use of this plant in other countries has not been evaluated. Thus, it is important to develop methods to screen different grades of glycerin for toxic JFs and other PEs.

Previously we have published a screening method for toxic phorbol esters in jerky pet treat products using a targeted MRM analysis [7]. The objective of this study was to develop UHPLC-HRMS analytical methods to screen different grades of glycerins for JFs, other PEs and their degradants.

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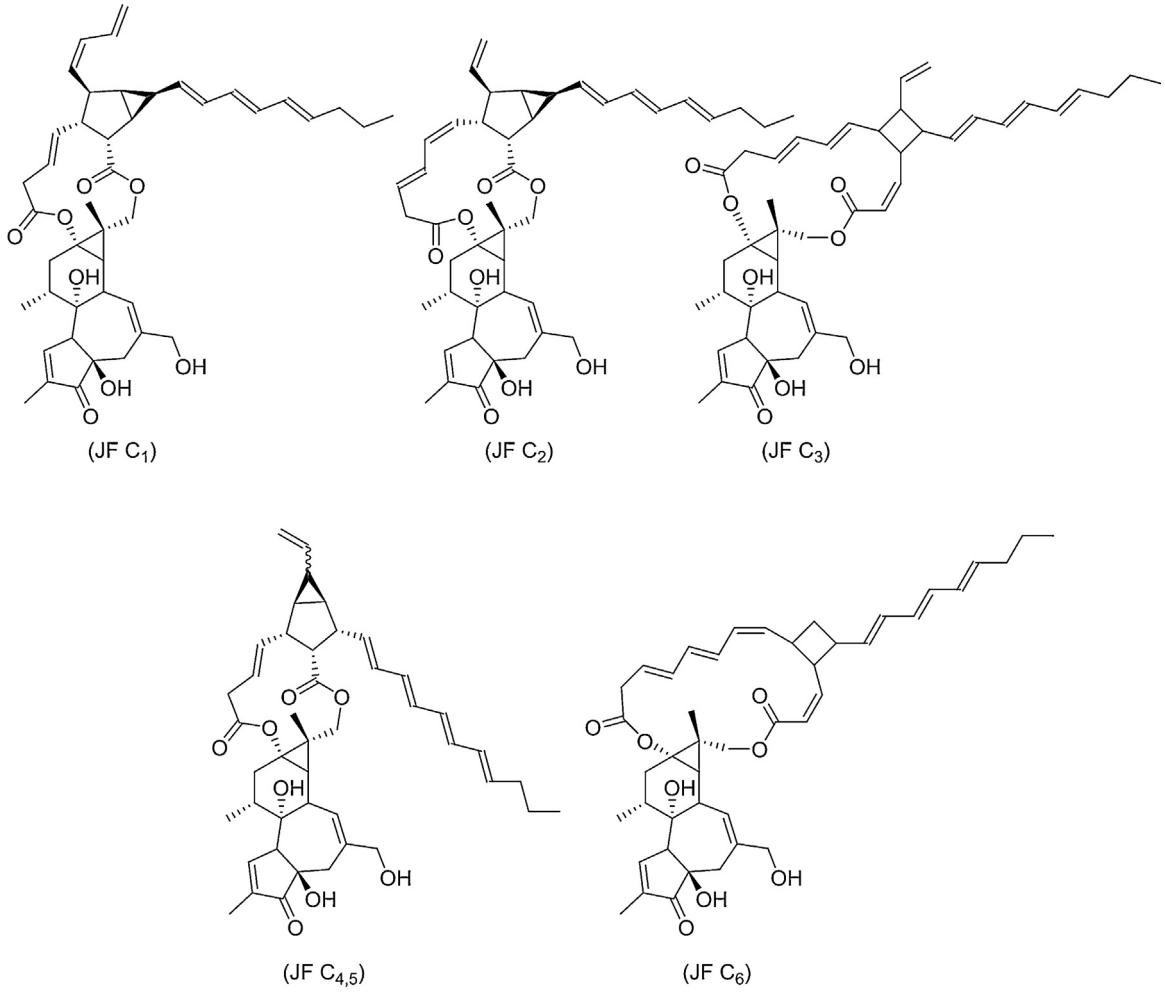

Fig. 1. Structures of isobaric JFs C_1–C_6 ($C_{44}H_{54}O_8$; 710.3813).

2. Materials and methods

2.1. Materials

2.1.1. Instrumentation

Samples were analyzed using an Agilent 1290 Infinity UHPLC/6550 Q-TOF mass spectrometer (Agilent Technologies, Palo Alto, CA) employing positive-ion monitoring. Preparative HPLC was performed using Shimadzu Prominence LC-20AP Preparative LC (Shimadzu Corporation, Japan) with photo diode array detector. Sample evaporation was carried out using Savant SPD121P Speed-Vac Concentrator (Thermo Fisher Scientific, Waltham, MA). The UV quantitation was performed using Agilent 1290 UHPLC with DAD detector (Agilent Technologies, Palo Alto, CA).

2.1.2. Chemicals

Phorbol 12-myristate 13-acetate (PMA), 4-α−phorbol and resiniferatoxin from Sigma Chemical Co. (Saint Louis, MO, USA) were used as standards. Methanol (MeOH), acetonitrile (ACN), and water were LC–MS grade (Burdick & Jackson, Muskegon, MI). Formic acid (LC–MS grade) from Fisher Scientific (Fair Lawn, NJ) was used in the preparation of LC mobile phases.

2.1.3. Plant materials

The seeds of *Jatropha curcas* were collected from China (Shen Yudian). The seeds were stored in a sealed bag in a dark, dry area.

2.1.4. Glycerin samples

Star KPO food- grade glycerin was obtained from Procter & Gamble (Cincinnati, OH), three crude glycerin samples were obtained from various sources and identified as grade 1, 2, and 3.

2.2. Preparation of Jatropha factors (JFs) standard from Jatropha curcas seeds

2.2.1. Extraction

Dried *Jatropha curcas* seeds (50 g) seeds were ground by mortar and pestle. Methanol (200 mL) was added to the ground seeds and stirred with a magnetic stir bar at room temperature for 10 min. The solution was then filtered under vacuum through a sintered funnel. Additional methanol (100 mL) was added to the extract after filtration for dilution. Methanol extract was defatted twice with hexane (200 mL) in a separatory funnel. Combined defatted methanol extract was evaporated using a SpeedVac concentrator to yield a residue (∼2.0 g) containing JFs. This residue was stored in a −10 °C freezer until used for further purification.

2.2.2. Purification of Jatropha curcas seed extract by preparative LC

A 70 mg aliquot of the residue from the methanol extract of *Jatropha* seeds was further purified by using Reversed-Phase Preparative HPLC (Luna C-18 column 250 × 10 mm, 10 μm particle size; Phenomenex, Torrance, CA, USA). Chromatographic separation was achieved by using a flow rate of 10 mL/min with a step