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Quantitative analyses of ricinoleic acid and ricinine in *Ricinus communis* extracts and its biopesticides

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Abstract The quantitative analytical method for the bioactive substance, 3-cyano-4-methoxy-*N*-methyl-2-pyridone (ricinine) and an index compound, ricinoleic acid in castor plant (*Ricinus communis*) extract or oil was developed. For the determination of a pyridone alkaloid compound, ricinine, successive cartridge clean-up method combined with ultra-performance liquid chromatography was set up with ENVI-Carb^{TM} (0.5 g) and C_{18} SPE cartridges. Accuracy and precision were evaluated through fortification studies of one biopesticide (PE) at 10 and 100 mg kg^{-1}. Mean recoveries of ricinine were 98.7 and 96.0 % associated with less than 10 % RSD, respectively. For the determination of ricinoleic acid in castor extract and oil, saponification and methylation were optimized using gas chromatography-time of flight mass spectrometry. Recovery was more than 84.8 % associated with 6.2 % RSD after derivatization procedure. Both methodologies developed were applied to analyze real samples including three castor oil products and six commercially available biopesticides containing *R. communis*, collected at Korean market. The contents of ricinine and ricinoleic acid in most commercial biopesticides were less than the oil or extract contents indicated by label.

Keywords 3-Cyano-4-methoxy-N-methyl-2-pyridone · Biopesticide · Quantitative analysis · Ricinoleic acid · *Ricinus communis*

Geun Hyoung Choi and Leesun Kim contributed equally to this work.

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Introduction

The synthetic pesticides have played a significant role in increasing food production. Large amount of the pesticides were manufactured and widely used since the 1940s (Oerke 2006). However, the long-term application of agrochemical pesticides has caused a serious negative impact on human health and the environmental safety (Damalas and Eleftherohorinos 2011). Therefore, the investigation about biopesticides as alternatives to synthetic pesticides has been actively performed by researchers since they are known to have very limited toxicity to the ecosystem and break down quickly in the environment (Wachira et al. 2014; Mansour and Abdel-Hamid 2015; Sabatino et al. 2015). In Korea, a variety of plant extracts or oil, known as biopesticides, are available in the markets without indications of the bioactive compounds or the target pests. However, not many analytical methods to screen and determine the bioactive compounds in biopesticides are available for quality control (Lim et al. 2014a). The rapid and sensitive analytical method for determining 3-cyano-4-methoxy-*N*-methyl-2-pyridone (ricinine) and ricinoleic acid (a main component for castor plant extract and castor oil) should also be established for quality control of commercial biopesticides containing the castor plant (*Ricinus communis* L.) extract or oil. The structures of both analytes are shown in Figs. 1A and B.

Ricinine is known as a toxic alkaloid present in *R. communis* L. (Li et al. 2013). As a biomarker for ricin, major toxin from *R. communis* L., ricinine was determined using different analytical techniques including gas chromatography mass spectrometry, liquid chromatography (LC)-MS and LC-MS/MS (tandem mass spectrometry) in a wide range of research (Coopman et al. 2009; Li et al. 2013). However, castor plant extracts or ricinine alone were also evaluated for their bioactivity against agricultural pests in some research. Bullangpoti et al. (2011) demonstrated that as a bioactive compound, ricinine may be used as an alternative for the minimal application of chemical insecticides for *Spodoptera*

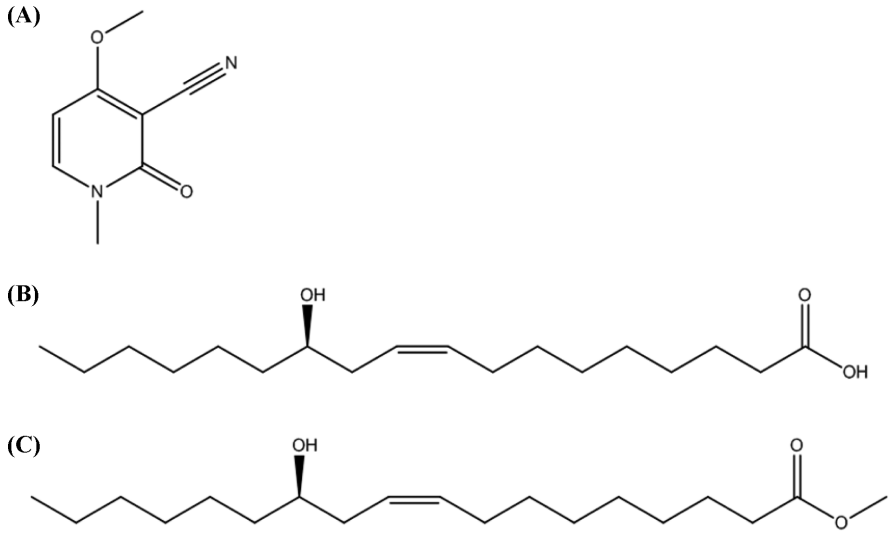

Fig. 1 The structures of (A) ricinine, (B) ricinoleic acid and (C) methyl ricinoleate

exigua. Wachira et al. (2014) found that out of six plants, extracts from *Tithonia diverifola* and *R. communis* showed the highest bioactivity against females of *Anopheles gambiae s.s.* acting as malaria vector. Extracted from *R. communis*, ricinine alone also showed toxicity similar to the plant extract against the same mosquito (Wachira et al. 2014). Some commodities containing castor plant extract or oil available at the Korean market are used as biopesticides in farming without the knowledge of its insecticidal effect.

This study was designed to develop an efficient and selective clean-up method for the determination of ricinine in biopesticides using ultra performance liquid chromatography (UPLC). After optimization of saponification and esterfication, ricinoleic acid was analysed as methyl ricinoleate to quantify the most abundant fatty acid in oil products and commodities containing castor oil using gas chromatography-time of flight mass spectrometry (GC-TOFMS). Finally, the developed methodologies were applied for the determination of two target analytes in the castor oil products and commodities (containing castor extracts or oil) collected at the Korean market.

Materials and Methods

Chemicals and reagents

Ricinine (purity 99 %) and Ricinoleic acid (purity 99 %) were purchased from Accurate chemical & Scientific Corp. (Westbury, NY, USA) and Sigma-Aldrich (Saint Louis, MO, USA) respectively. high performance liquid chromatography-grade acetonitrile (MeCN), methanol (MeOH), isooctane, sodium hydroxide (NaOH) were purchased from Merck (Darmstadt, Germany). ENVI-Carb^{TM} SPE cartridges (0.25 g, 3 mL and 0.5 g, 6 mL) were purchased from Supelco (Bellefonte, PA, USA). C_{18} SPEs (0.5 g, 6 mL) were purchased from Phenomenex (Torrance, CA, USA). Boron trifluoride (BF_3, 14 % in MeOH) was from Sigma-Aldrich. Three castor oil products and six commercial biopesticides containing *R. communis* were collected from the Korean market and stored in a refrigerator (4 °C).

Solid phase extraction (SPE) clean-up for ricinine

ENVI-Carb^{TM} SPE cartridge (0.5 g) and C_{18} SPE cartridge selected based on the previous study (Lim et al. 2014b) were activated with 3 mL of MeOH and 3 mL of deionized water (DW) respectively before sample loading. The sample (1 mL) was diluted with DW (10 to 100 fold) and then loaded on ENVI-Carb^{TM} SPE cartridge (0.5 g). After the sample was left for 10 min, the cartridge was washed with 3 mL of DW four times. And then, C_{18} SPE cartridge was placed on the bottom of ENVI-Carb^{TM} cartridge and target compound was eluted with 3 mL of MeOH four times. The eluate was concentrated with nitrogen (N_2) gas stream and re-dissolved in MeOH (1 mL) for UPLC analysis.

Fortification studies

The fortification studies of ricinine were performed with one plant extract (PE) commercially available, the product with surfactant (20 %, Tween 20) and DW as a blank. PE was fortified with ricinine standard at 10 and 100 mg kg^{-1}. PE was used as a control sample. The commodity containing surfactant (diluted 10 to 100 fold) and DW were fortified at 10 mg kg^{-1}.

Methylation of ricinoleic acid

Derivatization method of ricinoleic acid was modified from fatty acid analysis method developed by Korean Food Standards Codex. Isooctane (1 mL) was added to ricinoleic acid standard (10 mg) and the oil sample (20 mg) in a glass tube. For saponification, methanolic NaOH (0.5 N) was added to the sample and N_2 gas

Table 1 The gradient method for UPLC analysis of ricinine

Time (min)	Solvent A† (%)	Solvent B‡ (%)
0	95	5
5	90	10
10	70	30
15	0	100
18	0	100
20	95	5
22	95	5

†Solvent A: deionized water with 0.05 % formic acid; ‡Solvent B: methanol with 0.05 % formic acid

was filled into the tube, covered with a cap. And then the sample was heated at 100°C for 5 min for reaction. For esterification, isooctane (1 mL), BF_3 (14 %) and N_2 gas were added to the sample cooled down from the first reaction and shaken vigorously for 30 s. And then, saturated saline solution (5 mL) and N_2 gas were added to the sample and shaken for 30 s. After being cooled down, the solvent layer was collected and concentrated. The target analyte was redissolved with dichloromethane (5 mL) for the GC-TOF MS analysis.

UPLC instrumentation

Ricinine was analyzed on a Waters UPLC system with TUV detector. The column was a Waters Xbridge™ C_{18} (3.5 μm particle size, 3×150 mm). The injection volume was 5 μL and the flow rate was at 0.2 mL min^{-1}. Detection wavelength for the target compound, ricinine was 313 nm. The UPLC was operated using a gradient mobile phase of solvent A (0.05 % (v/v) formic acid in DW or DW) and B (0.05 % (v/v) formic acid in MeCN or MeCN). The gradient was shown in Table 1.

GC-TOFMS instrumentation

Methyl ricinoleate, derivatized from ricinoleic acid was analyzed on a GC system (7890A, Agilent Technologies, Santa Clara, CA, USA) coupled to TOFMS (Pegasus HT, LECO Corp. Saint Joseph, MI, USA) and an autosampler (7693 Agilent Technologies). Target analytes were separated on SP-2330 column (30 m × 0.25 mm i.d. × 0.2 μm d_f). The initial oven temperature was 100°C (held for 3 min) and was ramped to 240 °C with 20 °C min^{-1}. Carrier gas was helium at a constant flow of 1.0 mL min^{-1}. Temperatures of injector, transfer line and ion source were set at 240, 200 and 230 °C, respectively. The mass spectrometer was operated in the electron impact mode at 70 eV of electron energy. Mass spectra were recorded in the range of *m/z* 50–500.

Method validation for ricinine and ricinoleic acid

The method efficiency, expressed as recovery rates and relative standard deviation (% RSD) of the target analyte, ricinine was determined at two fortification levels: 10 and 100 mg kg^{-1} in spiked samples of one biopesticide (PE). In order to determine linearity for the UPLC analysis, a series of working solutions (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 mg L^{-1}) were prepared with ricinine standard (R^2 =0.9996). The repeatability (intraday RSD) of the replications on real samples was used to measure the precision of the method. The repeatability was established with three (n =3) complete analyses of each sample for both ricinine and ricinoleic acid under the same conditions in one day.

Results and Discussion

Optimization of clean-up procedure for ricinine

For the UPLC analysis, solid phase extraction (SPE) procedure was adopted for a rapid clean-up step to purify ricinine from the biopesticides containing *R. communis* L. or castor oil. ENVI-Carb™ and C_{18} SPE cartridges were selected as clean-up SPE cartridges since Lim et al. (2014b) demonstrated that the successive cartridge clean-up method (ENVI-Carb™ and C_{18} SPE cartridges) effectively removed interferences to purify matrine and oxymatrine out of biopesticides containing *Sophora falvescens* extract. Considering that marine and oxymatrine are also quinolizidine alkaloids, the pyridone alkaloid, ricinine was regarded to be adsorbed to the ENVI-Carb™ absorber and recovered with MeOH. Once the target analyte, ricinine was absorbed into ENVI-Carb™ cartridge. The cartridge was washed with deionized water (12 mL) to remove the surfactant since commercial biopesticides generally contains a surfactant as additive. The UPLC analysis demonstrated that good recoveries (75–99 %) associated with <10 % RSD for two spiking levels with one sample (PE) were achieved (Table 2).

Selection of column and mobile phase for ricinine

Waters Xbridge™ C_{18} (3.5 μm particle size, 3×150 mm) was

Table 2 Recoveries obtained from fortification studies of ricinine at 10 and 100 mg kg^{-1} with RSDs <10 % for plant extract sample, deionized water, and surfactant product using mobile phase with and without acid

Untreated samples	Spiking level (mg kg^{-1})	Mobile phase without acid		Mobile phase with acid	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
DW (Control)	10	84.7	5.4	91.1	7.0
20 % Tween 20*	10	90.8	0.5	91.5	1.2
PE#	10	57.4	1.4	98.7	3.7
	100	97.1	3.5	96.0	5.6

* Tween 20: surfactant product (20 % content of surfactant) # PE: Plant Extract

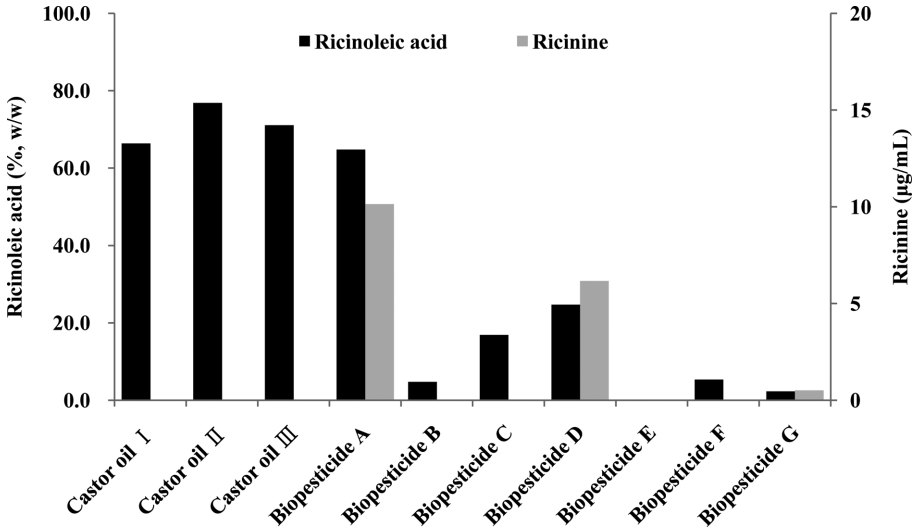

Fig. 2 Ricinoleic acid and ricinine contents in castor oil (I; reagent grade, II; produced in Korea, III; imported from India) and biopesticides commercially available at Korean market

selected, since the column was widely used for separation of ricinine in many studies (Coopman et al. 2009; Hamelin et al. 2012; Røen et al. 2013; Cai et al. 2014). The chromatogram obtained showed that the C_{18} column gave an excellent symmetrical peak for ricinine, thus C_{18} column was used for the determination of ricinine. One biopesticide (PE) not containing ricinine or castor oil was selected for fortification study since they had similar matrix to the biopesticide commodities containing *R. communis* L.. MeOH and DW, with and without 0.05 % formic acid were compared as a mobile phase since the acid is generally used for improvement of the chromatographic peak shape and to provide a source of protons in reverse phase. With formic acid, the mobile phase gave better peak shape and achieved consistent recoveries (shown in Fig. 2) for this study. The average recoveries for PE, 20 % tween and deionized water (as a control), were more than 90 % (<10 % RSD).

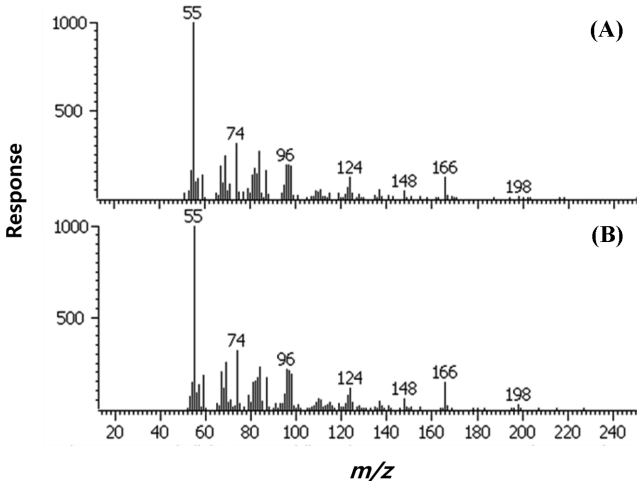

Fig. 3 Mass spectra obtained from (A) methyl ricinoleate standard and (B) methyl ricinoleate derivatized from ricinoleic acid

Quantification of ricinoleic acid

In a wide range of research, plant oils were screened for pest management (Don-Pedro 1989; Obeng-Ofori 1995; Haghtalab et al. 2009). Haghtalab et al. (2009) demonstrated that castor, hazelnut and other vegetable oils caused substantial mortality of *Callosobruchus maulatus* and castor oil provided better control of *C. maulatus*. It was suggested that insect death caused by oils is due to anoxia or interferences in normal respiration causing suffocation (Don-Pedro 1989). Considering that some biopesticides indicated to have castor oil or plant extract in Korea, it is also important to determine oil contents in those biopesticides products. Since more than 80 % of the fatty acid in castor oil is known to be ricinoleic acid (Akpan et al. 2006; Salimon et al. 2010), this fatty acid can be used as an index compound for oil contents in the biopesticides. In order to quantify ricinoleic acid, the methylation of BF_3 selected based on the fatty acid method developed by Korean Food Standards Codex, was optimized. For saponification and esterification procedures, reaction time (at 100 °C) was optimized. Recoveries obtained from different reaction times were firstly compared with 5 (saponification) and 40 (esterification) min and 10 (saponification) and 40 (esterification) min, respectively. Recoveries obtained from both methods were 30.9 with RSD 12.7 % and 28.6 with RSD 5.4 %, respectively. It was considered that the target analyte was lost due to the long reaction time. Therefore, reaction time was adjusted into 5 min for saponification and 2 min for esterification based on Kim et al. (2015). The average recovery of 84.8 % was achieved. This method was used for the analysis of the biopesticide samples.

Qualitative and quantitative mass ion to determine methyl ricinoleate derived from ricinoleic acid was observed. The structure of methyl ricinoleate was shown in Fig. 1C. The mass spectrum obtained from methyl ricinoleate derived from ricinoleic acid showed that the peaks were at 198, 166, 148, 124, 96, 74 and 55 *m/z* (Fig. 3B). The prominent peaks were at 166, 96, and 74 *m/z*. This was confirmed with mass spectrum obtained from the methyl ricinoleate standard (Fig. 3A) since they have the same fragmentation patterns. Out of the major peaks, the peak at 166 *m/z* was used for quantification of methyl ricinoleate since determination of coefficient using the peak at 166 *m/z* (R^2>0.9997, n=3) demonstrated good linearity compared with other ions (R^2<0.9997, n=3).

Quantification of ricinine and ricinoleic acid in commercial biopesticide

Using the developed methods, ricinine and ricinoleic acid were determined in reagent castor oil; two castor oil products originated from Korea and India, and seven commercial biopesticides (A-G) at Korean market. The analysis of results showed that oil from Korea, India and reagent contained 76.9, 71.1 and 66.4 %, respectively. On the other hand, ricinine was not detected at all from three castor oil samples. Since traditionally, castor oil can be used for medicine, hence it should not contain toxic compounds including ricin or ricinine (Cai et al. 2014). The oil products demonstrated were not to be contaminated. The LC results showed that only three samples (A and D) contained ricinine (10.1, 6.2 and 0.5 % (w/w) for each sample). While sample A, C and D contained 64.8, 16.9 and 24.7 % (w/w) of ricinoleic acid respectively, the rest of samples contained less than 5.3 % of ricinoleic acid (Fig. 2). The data showed that the contents of ricinine and ricinoleic acid were less than the contents of oil or extract indicated on the label of the products.

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References

Akpan UG, Jimoh A, Mohammed AD (2006) Extraction, characterization and modification of castor seed oil. Leonardo J Sci 8: 43–52

Bullangpoti V, Khumrungsee N, Pluempanupat W, Kainoh Y, Saguanpong U (2011) Toxicity of ethyl acetate extract and ricinine from Jatropha gossypifolia senescent leaves against *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). J Pestic Sci 36: 260–263

Cai M, Chen X, Wei X, Pan S, Zhao Y, Jin M (2014) Dispersive solid-phase extraction followed by high-performance liquid chromatography/tandem mass spectrometry for the determination of ricinine in cooking oil. Food Chem 158: 459–465

Coopman V, De Leeuw M, Cordonnier J, Jacobs W (2009) Suicidal death after injection of a castor bean extract (*Ricinus communis L.*). Forensic Sci Int 189: e13–e20

Damalas CA, Eleftherohorinos IG (2011) Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Heal 8: 1402–1419

Don-Pedro KN (1989) Mechanisms of action of some vegetable oils against *Sitophilus zeamais motsch* (Coleoptera: Curculionidae) on wheat. J Stored Prod Res 25: 217–223

Haghtalab N, Shayesteh N, Aramideh S (2009) Insecticidal efficacy of castor and hazelnut oils in stored cowpea against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J Biol Sci 9: 175–179

Hamelin EI, Johnson RC, Osterloh JD, Howard DJ, Thomas JD (2012) Evaluation of ricinine, a ricin biomarker, from a non-lethal castor bean ingestion. J Anal Toxicol 36: 660–662

Kim TW, Kim KK, Kang YH, Kim DJ, Lee JI, Choe M (2015) Ananalysis of seed oil fatty acids and their effect on lipid accumulation and leptin secretion in 3T3-L1 adipocytes. Korean J Food Sci Technol 47: 103–110

Li J, Gu D, Liu Y, Huang F, Yang Y (2013) Large-scale separation of ricinine from a by-product of *Ricinus communis* L. by pH-zone-refining counter-current chromatography. Ind Crop Prod 49: 160–163

Lim SJ, Jeong DY, Choi GH, Park BJ, Kim JH (2014b) Quantitative analysis of matrine and oxymatrine in *Sophora flavescens* extract and its biopesticides by UPLC. J Agric Chem Environ 3: 64–73

Lim S-J, Lee J-H, Kim JH, Choi GH, Cho N-J, Park BJ (2014a) Determination of dimethyl disulfide, diallyl disulfide, and diallyl trisulfide in biopesticides containing *Allium sativum* extract by gas chromatography. Korean J Environ Agric 33: 237–243

Mansour SA, Abdel-Hamid NA (2015) Residual toxicity of bait formulations containing plant essential oils and commercial insecticides against the desert locust, *Schestocerca gregaria* (Forskal). Ind Crop Prod 76: 900–909

Obeng-Ofori D (1995) Plant oils as grain protectants against infestations of *Cryptolestes pusillus* and *Rhyzopertha dominica* in stored grain. Entomol Exp Appl 77: 133–139

Oerke EC (2006) Crop losses to pests. J Agric Sci 144: 31–43

Røen BT, Opstad AM, Haavind A, Tønsager J (2013) Serial ricinine levels in serum and urine after ricin intoxication. J Anal Toxicol 37: 313–317

Sabatino L, Scarangella M, Lazzaro F, Scordino M, Picariello G, Leotta C, Traulo P, Gagliano G (2015) Matrine and oxymatrine in corroborant plant extracts and fertilizers: HPLC/MS-MS method development and single-laboratory validation. J Environ Sci Health B 50: 862–870

Salimon J, Noor A, Dina, Nazrizawati AT, Mohd Firdaus MY, Noraishah A (2010) Fatty acid composition and physicochemical properties of Malaysian castor bean *Ricinus communis* L. seed oil. Sains Malays 39: 761–764

Wachira SW, Omar S, Jacob JW, Wahome M, Alborn HT, Spring DR, Masiqa DK, Torto B. (2014) Toxicity of six plant extracts and two pyridone alkaloids from *Ricinus communis* against the malaria vector *Anopheles gambiae*. Parasit Vectors 7: 312