S & D SHORT COMMUNICATION

Rapid Detection of Argemone Oil in Rapeseed/Mustard Oils with a Pressurized Mini-Column

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A simple, rapid, reliable and economical method for the detection of argemone oil contamination in edible oils was developed. The method employs a commonly available glass syringe which is tightly packed with certain adsorbents. Sanguinarine which is used as an index of argemone oil contamination in edible oils is detected as a compact golden yellow fluorescent band under long wave UV light after chromatographic development. The pressurized mini-column (PMC) method is capable of detecting sanguinarine contamination at a 500 ppb level.

Rapeseed/mustard oil (*Brassica* sp.) is consumed by population groups in countries of Asia, Europe and North America (1). In India, rapeseed/mustard oil is occasionally contaminated with argemone oil. The contaminated oil poses a health hazard to the consumers (2,3) and in some cases has caused death from cardiac failure. The toxic principle was identified by Sarkar (4) to be an alkaloid, sanguinarine. Recently, it has been applied in toothpaste and oral rinse as an antiplaque agent (5). In view of its known toxic effects (4,6), it is essential to monitor its levels both in oral preparations and edible oils in India where contamination with argemone oil is likely.

Several chemical, chromatographic, colorimetric and spectrophotometric methods have been developed for the detection of sanguinarine as an index of argemone oil contamination in edible products (7–15). Most of these methods are time-consuming. There is a need for a method applicable in the field for routine surveillance. This paper describes a simple, rapid, economical and reliable procedure for the detection of argemone oil contamination in mustard oil.

MATERIALS AND METHODS

Glass syringes of borosilicate glass (5 mL and internal diameter 11 mm) were obtained from Hindustan Syringes, India. Florisil (mesh 100–200) was procured from Sigma Chemicals, St. Louis, MO. Column grade silica gel (mesh 60–120) was purchased from Acme Synthetic Chemicals, Bombay, India. Pure sanguinarine standard was prepared in the laboratory from argemone oil by extracting it into 6N Hcl:Methanol (1:1). Sanguinarine was further purified to homogeneity by preparative thin-layer chromatography. All other chemicals used were of analytical reagent grade.

Preparation of mini columns. Syringes were fitted with Whatman No. 4 filter paper disc of the same internal diameter to hold the adsorbents. The syringes were packed in the following order: a layer of anhydrous sodium sulphate to a height of 2 mm; layer of 5 mm thick silica gel; and layer of 2 mm thick Florisil. Another filter paper disc was placed on the Florisil layer and was tightly packed after the addition of a 4 mm layer of anhydrous sodium sulphate with the aid of a glass rod. After the completion of the packing, the column was activated at 110°C for 1 hr in an oven.

Extraction of sanguinarine. Pure mustard oil was spiked with pure argemone oil at 0.01%, 0.1%, 0.5%, 1.0%, 1.5% and 2.0% levels and the sanguinarine was extracted with methanol:6N Hcl (1:1). Genuine argemone oil was found to contain 5.4 mg/sanguinarine mL (13). In a typical experiment, 2 mL of spiked sample was mixed thoroughly with 2 mL of methanol:6N Hcl (1:1) reagent. This reaction mixture was placed in a boiling water bath for about 2 min so as to break the emulsion. The lower aqueous layer (1 mL) was used for the detection of sanguinarine. Same procedure was repeated by spiking mustard oil with standard sanguinarine at 5.4 μg/mL and 54 μg/mL respectively, and the efficiency of extraction was found to be 80% (CV 5%) which was analyzed fluorimetrically (13).

Detection and quantitation. The lower aqueous layer of the extract was loaded onto the activated column and was allowed to drain by gravity. Immediately 3 mL of solvent (chloroform:hexane, 6:4) was pressurized through the column with the plunger. Later this column was observed under a long wave UV (366 nm) source for the presence of characteristic golden yellow fluorescence of sanguinarine. The fluorescent band appeared at the junction of the Florisil and silica gel layers.

Quantification of sanguinarine in the contaminated samples was done by comparing the intensity of the fluorescent band of the column developed with that of standard sanguinarine solutions. A correction factor (C) of 1.25 was used in the calculation of sanguinarine (μg/mL) in the sample. The procedure is similar to the one described for aflatoxins (16,17).

RESULTS AND DISCUSSION

Sanguinarine gives a characteristic golden yellow fluorescence under long range UV (366 nm). This property has been used in the detection of argemone oil contamination. Florisil specifically adsorbs sanguinarine; thus, only a single bright golden yellow fluorescent band was observed under UV. Due to compact packing of the column, sanguinarine appears as a sharp fluorescent band without any dispersion. Table 1 gives the analysis of sanguinarine in mustard oil by PMC and TLC fluorimetric methods. The levels of argemone oil spiked in the present study were based on the natural outbreak reported by Shenolikar *et al.* (18) where the edible oil was found to be contaminated at 0.16% to 2.2%. Analysis of spiked samples by PMC method and TLC fluorimetric method shows that the variation between the methods ranged from 17 to 33%. The coefficients of variation with respect to repeatability and reproducibility of the method within the laboratory at two levels of spiking (0.1 and 1% argemone oil) were 22% and 31% respectively. The

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TABLE 1

Comparison of PMC/Visual Method with TLC/Fluorimetric Method

Sample[a]	Percentage (%) of argemone oil spiked to mustard oil	Sanguinarine μg/ml		(%) Variation of PMC-visual method
		TLC/Fluorimetry	PMC/Visual method	
1	0.01	0.54 (10.7)[b]	<0.50	—
2	0.1	5.4 (7.4)	4.5	17
3	0.5	27.0 (6.3)	22.5	17
4	1.0	54.0 (5.5)	45.0	17
5	1.5	81.0 (5.2)	60.0	26
6	2.0	104.0 (4.9)	70.0	33

[a] All samples were analyzed in triplicates.

[b] Coefficient of variation.

detection limits by PMC and TLC fluorimetric method were 500 ng/mL and 200 ng/mL, respectively.

The method developed is simple, rapid and economical as compared to all the earlier methods reported. The entire process of the sample extraction and detection of the contaminant takes about 15 min, unlike the TLC method which takes about 4 hr. The advantage of the present method is that it can also be successfully used at a field level for rapid screening of edible oils for the contamination of argemone oil and, as a quality tool, as a means for monitoring sanguinarine levels in commercial oral health care preparations.

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