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A Rapid Technique for Detection and Quantification of Mineral Oil in Vegetable Oils Used As Vehicles in Ayurvedic Formulations

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ABSTRACT
The study was conducted to check the purity of vegetable oil used in the preparation of ayurvedic oil preparations using HPTLC fingerprinting. Adulteration of vegetable oil is usually by using mineral oils. The proposed TLC/ HPTLC method was found to be simple, rapid, accurate and reproducible for the identification and estimation of mineral oil in various vegetable oils and its finished formulations.

Keywords: Vegetable oil, adulteration, mineral oil, HPTLC finger printing.

1. Introduction
In ayurveda about 62 formulations [1] are using different forms of vegetable oils as its vehicles. Some of them are for external and others for internal applications. Due to scarcity of vegetable oils, often it is adulterated with mineral oils. Mineral oils are listed as group 1 carcinogens to humans. It is also used as a lubricant in enema preparations. In this paper a simple TLC method was developed to identify the presence of mineral oil in various vegetable oils/ ayurvedic finished formulations and its quantification using HPTLC.

2. Materials and Methods
2.1 Chromatographic Conditions
Pre-coated silica gel plates Merck 60 F_{254} (20 X 10 cm, 0.2 mm thickness), Wavelength: 650 nm, CAMAG Automatic TLC Sampler 4, Twin Trough Chamber Scanner: TLC Scanner 3 and CATS software were used.

2.2 Preparation of Standard
A stock solution of mineral oil (1 mg/ml) was prepared by dissolving 10 mg of weighed sample in hexane and making up the volume up to 10 ml.

2.3 Calibration curve for Standard
The standard solution of mineral oil (5 µg to 25 µg per respective spot) was applied in triplicate on TLC plate. Quantitative evaluation of the plate was performed after developing with hexane and derivatization with anisaldehyde-sulphuric acid reagent[2] and scanning at 650 nm.

2.4 HPTLC Quantification in Test Samples
Sesame oil, coconut oil, castor oil containing 1, 5, 10, 25, 50 and 80 percentages of mineral oil was accurately prepared. 1g of each sample were accurately weighed and made up to 25ml using hexane. The plates were developed by ascending mode to a distance of 8 cm and scanned as per the conditions mentioned above. The mineral oil content was determined by comparing the area of the chromatogram with the calibration curve of working standard. The R_f value of the standard mineral oil (0.86) was compared with the R_f value of the extracts. TLC methods were followed as per the earlier reported procedure [2].

3. Results and Discussion
Different vegetable oils like coconut oil, castor oil, sesame oil were selected and tested for adulteration with mineral oil using standard methods reported in literature Samples of the above vegetable oils were added with 1%, 5%, 10%, 50%, 80% mineral oil. From the samples 1 g weighed and made up to 25 ml in a standard volumetric flask using hexane.

From this stock solutions 20, 10, 5, 1µl of the solutions were applied respectively on a TLC plate and various mobile phases were tested and the desired resolution was achieved by using hexane. Simple TLC of oils clearly reveals the presence of mineral oils in the samples after derivatization with anisaldehyde-sulphuric acid reagent followed by heating at 110 ºC for 10 min without charring (Fig-1). A commercial sample reported to contain 79.7% mineral oil was tested using the method and it clearly depicts the presence of mineral oil. Five formulations were analysed and it found to be free from mineral oils. For the quantification calibration curve of mineral oil was obtained by plotting peak areas verses concentration applied. It was found to be linear in the range of 5 µg to 25 µg per spot. Equation of the calibration curve is y = 164.31 + 7.926x. The correlation coefficient was found to be 0.99240 and thus exhibits good linearity between concentration and area (Fig-2). The results of recovery study are given in Table 1. For mineral oils, relative standard deviation of all the parameters is less than 2 % for the degree of repeatability indicating the high repeatability of the proposed method.

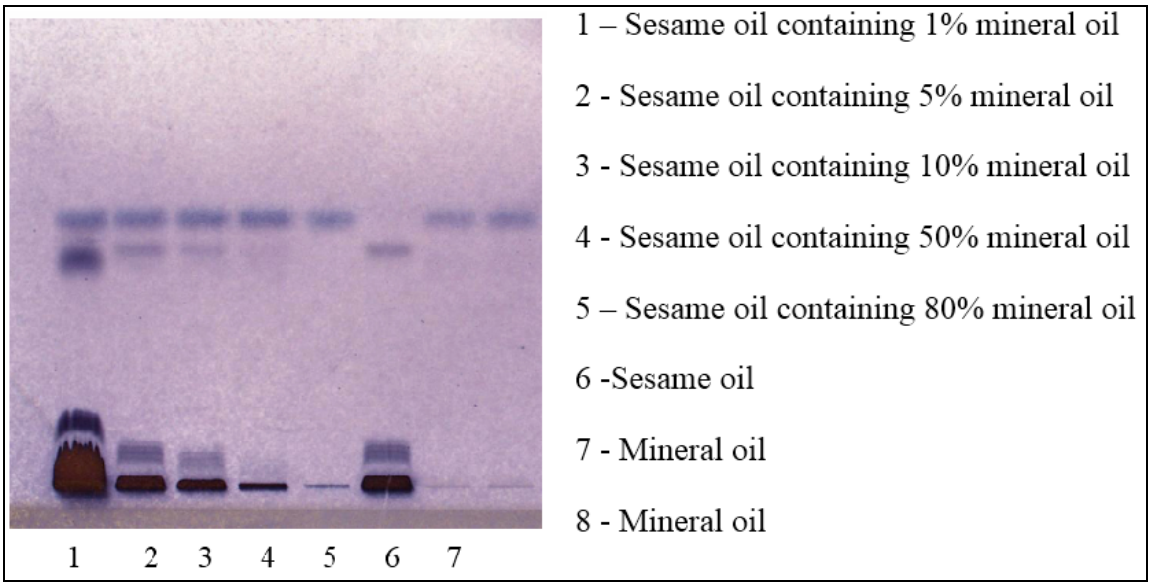

Fig 1: TLC Profile of the samples

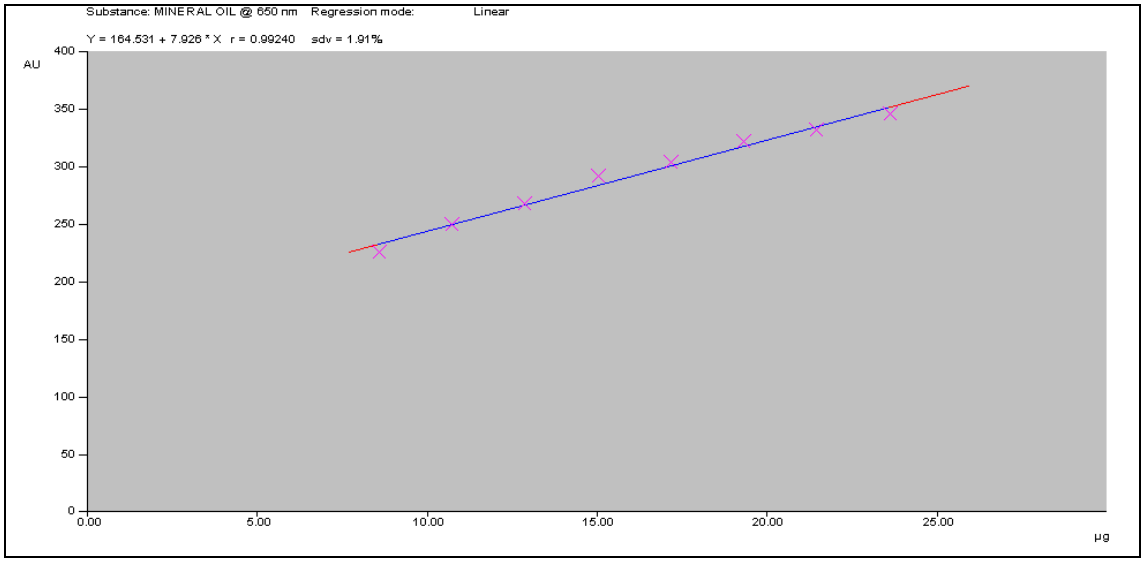

Fig 2: Linear calibration curve of the mineral oil.

Table 1: Recovery study of Mineral oils in Vegetable oils by HPTLC.

Theoretical amount of mineral oil spiked (mg/ml)	Practical amount of mineral oil found (mg/ml)	% Recovery ± S.D. (n=3)
0.4	0.3894	97.46 ± 0.12
2	1.9264	96.32 ± 0.14
4	3.848	96.2 ± 0.12
20	17.846	89.23 ± 0.11
32	28.2048	88.14 0.15

4. Conclusion

In conclusion, the proposed TLC/ HPTLC method was found to be simple, rapid, accurate and reproducible for the identification and estimation of mineral oil in various vegetable oils and its finished formulations.

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