Combined Thin-Layer Chromatography-Densitometry Method for the Quantitative estimation of Major Alkaloids in Poppy Straw Samples

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A simple, quick and inexpensive thin layer chromatography - densitometry method for the determination of major opium alkaloids morphine, codeine, thebaine, papaverine, and narcotine in poppy straw samples is described. The assay combines the separation of compounds in Silica gel 60 F_{254} thin layer plates with spot visualization by spraying plates with Dragendorff reagent and densitometry by dual wavelength absorption mode.

P. SOMNIFERUM is valued for analgesic and antispasmodic alkaloids such as morphine, codeine, thebaine, papaverine and narcotine. Although, TLC in combination with UV[1], spectrophotometer[2], colorimeter[3], or densitometer[4] is applied for the analysis of opium olkaloids, no report is available on the use of dual wave length TLC-densitometry: a rapid analytical procedure, for simultaneaous analysis of major opium alkaloids during a large scale screening of plant materials obtained in breeding and physiological experiments.

Densitometer used was a Desaga model CD-50, equipped with a dual wavelength thin layer chromatography scanner and data recorder. Precoated Silica gel plates 60-F_{254} with a layer thickness of 0.25 mm (Merck) were used. Known amounts of morphine, codeine, thebaine, papaverine and narcotine (1 mg/ml for each stock solutions) were prepared in methanol. Different amounts of the alklaoids were loaded on TLC plate (20x20cm). Chromatography was carried out in glass TLC tank saturated with the mobile phase toluene-acetone-methanol-ammonia (40:40:6:2) and the plates were developed to a height of about 15 cm. Plates were taken-off, dried and spots were visualized by immersing in Dragendorff reagent with tartaric acid[5]. For quantification of TLC spots, the chromatographic zones on the TLC plates, corresponding to morphine, codeine, thebaine, papaverine and narcotine were scanned at 530 nm and 650 nm using DWT mode with background subtraction and using a light spot of 1x2 nm. Calibration curves of these alkaloids were constructed by plotting concentration versus spot area. Air dried poppy straw (0.2 g) was extracted with methanol (3x10 ml) overnight. The extract was concentrated in vacuo, 1 ml of methanol was added and known quantity of this solution was spotted on TLC plate to calculate the percent content of alkaloids with the help of calibration curves. HPLC analysis of poppy straw for morphine, codeine, thebaine, papaverine and narcotine were performed using the method previously reported.[6]

The calibration curves for morphine, codeine, thebaine, papaverine and narcotine were linear in the range from 2 μg to 20 μg. The regression equations were y=35.819x-9.599 (r=1.00), y=12.300x-0.500 (r=0.99), r=5.729x-0.710 (r=0.99), y=29.142x+5.666 (r=0.99) and y=38x-0.800 (r=0.99) for morphine, codeine, thebaine, papaverine and narcotine, respectively. TLC using a mixture of toluene-acetone-methanol-ammonia (40:40:6:2) gave good resolution of these alkaloids (Fig.1). The average content of morphine (M), codeine (C), thebaine (T), papaverine (P) and narcotine (N) were 0.920, 0.045,

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Table 1 : Estimation of morphine, codeine, thebaine, papaverine and narcotine in poppy straw

Alkaloid	% content		TLC-DM
	TLC-Densitometry	HPLC	HPLC
Morphine	0.920	0.942	0.98
Codeine	0.045	0.046	0.98
Thebaine	0.036	0.038	0.95
Papaverine	0.048	0.050	0.96
Narcotine	0.045	0.047	0.96

0.036, 0.048 and 0.045% respectively and the coefficient of variation (C.V.) were 2.325, 6.667, 5.556, 4.167 and 4.969, respectively, in a poppy straw sample analysed by dual wave length TLC-densitometry (as above). For the examination of recovery rates, known amount of M,C,T,P and N were added in poppy straw and the quantitative analysis repeated thrice. Recoveries of M, C,T,P and N were found to be 96%, 95%, 95%, 98% and 96%, respectively. Quantitative determination of poppy straw samples both by TLC-densitometry and HPLC are shown in Table 1. Results are comparable to HPLC results. The method applied here is simple, inexpensive than HPLC (no use of HPLC grade solvents, HPLC columns) provides scanning of 15 plant extracts in a single 20x20 cm TLC plate in about 2 h time and is useful in the screening programme of a large number of plant samples in crop improvement experiments.

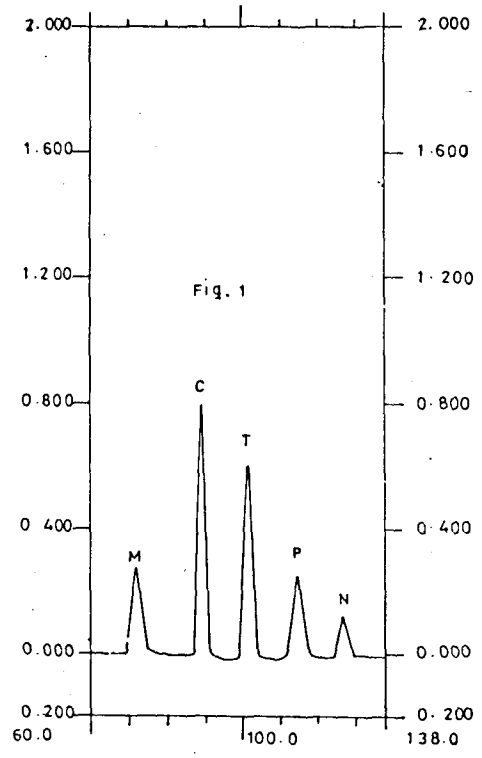

Fig. 1 : Scanning profile of Opium straw alkaloids using TLC- densitometry method. Absorbent : silica gel 60 F_{254}. Mobile phase : toluene-acetone-methanol-ammonia (40:40:6:2). Detection: Dual wave length detection (DWT) mode at 530 nm and 650 nm. M: morphine : C:codeine: T: thebaine: P: papaverine; N: narcotine.

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Niosomal Delivery of Tenoxicam

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The new trends in the development of drug delivery systems are based on the application of the concepts and techniques of targeting drugs to specific sites in the body using various carriers and vehicles as drug delivery devices[1,2]. This study describes the encapsulation of Tenoxicam in niosomes and investigates the influence of the varying proportion of surfactant, cholesterol and dicetyl phosphate on the morphology, particle size distribution, entrapment efficiency and *in-vitro* drug release of niosomes.

TENOXICAM was obtained from Ranbaxy Laboratories Ltd., New Delhi, Dicetyl phosphate, Sigma Chem. Co., St. Louis USA, Cholesterol from S.D. Fine Chem. Ltd., Mumbai Carrageenan, Indian Gum Industries Ltd. and Cellophane membrane from Kesavarm Rayon India.

The niosomes were prepared by hand shaking method as described by Baillie **et al**[3], and Azmin **et al**[4]. Different batches of niosomes were prepared using accurately weighed quantities of the Span 60/cholesterol/dicetyl phosphate respectively as molar mixture viz. B_1 (50:50), B_2 (60:40), B_3 (70:30), B_4 (80:20), B_5 (90:10), B_6 (47.5:47.5:5), B_7 (60:35:5), B_8 (Span 60 only 100 μmol), B_9 (Span 60 only 200 μmol).

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The lipid ingredients were dissolved in 10-15 ml of diethyl ether and transferred to a 50 ml round bottomed flask. The ether was removed at room temperature under reduced pressure on a rotary film evaporator (Buchi type) to form a thin dry film on the inner wall of the flask. The dried surfactant film was hydrated with 5 ml of aqueous phase at 60° be gentle agitation for one hour to obtain a milky dispersion of niosomes.

The aqueous phase used for film hydration was either 5 ml drug solution, (2 mg/ml of Tenoxicam) or 5 ml buffer solution to give drug loaded or empty niosomes respectively. All the batches of niosomes were triplicated.

The niosomes were examined under the optical microscope. The major proportion of niosomes were