J. Indian Chem. Soc.,
Vol. 84, January 2007, pp. 69-73

Search for suitable mobile phase in TLC analysis of different drugs of forensic interest and their gas liquid chromatographic experiment

Dilip Kumar Kuila[a] and S. C. Lahiri[b]*

[a]Central Forensic Science Laboratory, 30, Gorachand Road, Kolkata-700 014, India

E-mail : kuiladk@rediffmail.com

[b]Kalyani Government Engineering College, Kalyani-741 235, West bengal, India

E-mail : sujitclahiri@yahoo.com

Manuscript received 21 August 2006, accepted 7 November 2006

Abstract : Cannabis and related plant products from *Cannabis sativa* L. (THC, CBD, CBN), opium alkaloids from *Papaver somniferum* L. (morphine, narcotine, codeine, thebaine, papaverine etc.), cocaine, methaqualone, have been widely used and abused all over the world. These compounds are seized and sent by the law enforcing authorities to forensic science laboratories to detect and quantitate the different constituents contained in the illicit preparations.

Thin layer chromatography (TLC) is one of the most efficient method of detection of these compounds. Attempts were made to find out the suitable developing solvent systems for TLC analysis of constituents of cannabis, opium alkaloids, cocaine and methaqualone mentioned above. The results of this study will be helpful to (i) find the limit of detection and (ii) to make a comparative evaluation of different solvent systems for the analysis of the constituents of the above mentioned drugs. Efforts were also made to find out the retention time of some of the drugs in gas chromatographic technique.

Keywords : Cannabis products, TLC, GC, solvent system, comparative evaluation.

Cannabis and related plant products from *Cannabis sativa* L. [marijuana (THC 0.5–5%), hashish (THC 2–10%) and hashish oil (THC 10–30%), the main psychoactive constituents being tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN)][1-4], opium alkaloids obtained from the milky juice of unripe poppy (*Papaver somniferum* L.) seeds [the main constituents being morphine (4–21%), narcotine (2–8%), codeine (0.7–3%), thebaine (0.2–1%), papaverine (0.5–1.3%) and meconic acid (upto 15%)], cocaine (extracted from the leaves of the plant *Erythroxylon coca*) and related products like methqualone have been widely used and abused all over the world[2,3]. The compounds are of great forensic interest as most of the compounds are illicit in nature. Law enforcing authorities usually seize the clandestine preparations of these illicit compounds, distributed by a chain of drug smugglers and peddlers to drug addicts. The seized drugs are usually sent to different forensic laboratories including Central Forensic Science Laboratory, Kolkata to detect and quantificate the different constituents contained in the illicit preparations and find out the source or origin of the drugs to understand the route of drug trafficking.

A simple and low cost method widely practiced for the detection of these compounds is to use thin layer chromatography (TLC)[5-10]. The requirements for the improvement of the TLC method are :

(i) search for the suitable solvent system with better differentiating capabilities and

(ii) search for new chromogenic agents of high selectivity and specificity.

In the present study, attempts were made to find out the suitable developing solvent systems for TLC analysis of constituents of cannabis (THC, CBD, CBN), opium alkaloids (morphine, codeine, thebaine, papaverine, narcotine), cocaine and methaqualone. The results of this study will be helpful to (i) find the limits of detection and (ii) to make a comparative evaluation of the different solvent systems for the analysis of the constituents of the above mentioned drugs. Efforts were made to find out the retention time of some of the drugs in gas chromatographic technique.

Experimental

The compounds studied were arranged in three different groups –

(a) constituents of cannabis

(b) opium alkaloids

(c) cocaine and methaqualone

All the solvents used in the experiment (methanol, acetone, toluene, petroleum ether, diethyl ether, *n*-hexane, ethylacetate, cyclohexane, chloroform and ammonia) were of HPLC grade (EM, Germany).

The standardization of the solvent systems were made using standard sample of CBD, THC, CBN for cannabis products (0.5 mg/ml methanolic solutions), 1 mg/ml methanolic solution of morphine base, codeine base, thebaine base, narcotine base, papaverine base, heroine hydrochloride, cocaine, methaqualone.

Extraction of the contents from cannabis plant (ganja) : 1.0 g of pulverized cannabis plant was shaked with 20 ml toluene or petroleum ether for one hour. The contents were filtered and the residues were washed using same solvent. The filtrate was transferred into volumetric flask and made up to the mark with toluene or petroleum ether.

Extraction of the contents from opium : 0.3 g of dry opium powder was shaked with 50 ml of 5% acetic acid for 90 min. The contents were filtered and washed with 5% acetic acid. The filtrate was made alkaline (pH 9.2) using ammonia and the extraction of the contents was made with 30 ml of chloroform : iso-propanol mixture (3 : 1). The process was repeated three times. The extracted solution was dried over anhydrous sodium sulphate. The solvent was evaporated to dryness under vacuum. The contents were extracted with 2 ml of ethanol +5 ml of toluene and evaporated to dryness. The process was repeated twice. The residue was dissolved in 10 ml of methanol.

Spraying reagents were 50 mg Fast blue B salt (di-*o*-anisidine tetrazolium chloride) in 20 ml of 0.1 *N* NaOH for cannabis products and acidified potassium iodoplatinate (prepared by dissolving 0.25 g platinic chloride and 5 g potassium iodide in water with addition of 2 ml of conc. HCl and diluting to 100 ml) for opium alkaloids and cocoa products.

Solvents (Mobile phase) system used for TLC analysis are tabulated as[11-18]

Group of testing	Solvent system number	Solvent system	Ratios
Cannabis and related drugs	1	Toluene	100
	2	Petroleum ether : diethyl ether	80 : 20
	3	Petroleum ether : diethyl ether	50 : 50
	4	*n*-Hexane : toluene : diethyl amine	75 : 25 : 5
	5	*n*-Hexane : dioxane : methanol	70 : 20 : 10
Opium alkaloids	6	Ethylacetate : methanol : ammonia (25%)	85 : 10 : 5
	7	Cyclohexane : chloroform : diethyl amine	50 : 40 : 10
	8	Methanol : ammonia	55 : 45
Cocoa products	9	Ethylacetate : methanol : ammonia (25%)	100 : 20 : 1
	10	Cyclohexane : toluene : diethyl amine	75 : 15 : 10
	11	Methanol : ammonia (25%)	100 : 1.5

TLC Plates : The plates were 20 cm × 20 cm (E. Merck) pre-coated glass plate with silica gel 60 F_{254} of 0.25 mm layer thickness.

Samples were spotted on TLC plates by means of auto sampler and dried. The experiments were conducted by placing the plates in different solvent systems mentioned before and the colour of the spots on the plates were developed by spraying suitable chromogenic agents mentioned before.

Table 1. R_f values of CBD, THC, CBN for limit of detection (solvent system 1)

Drug	Colour	Volume spotted in µl											
		0.5	1	2	3	4	5	6	7	8	9	10	Av. R_f
CBD	Reddish brown	0.58	(0.57)	0.57	0.57	0.57	0.57	0.58	0.58	0.58	0.58	0.58	0.575
THC	Purple	(0.52)	0.51	0.51	0.51	0.51	0.52	0.54	0.54	0.53	0.55	0.55	0.526
CBN	Violet	(0.48)	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.47	0.47	0.47	0.477

Values in parenthesis are R_f for the minimum detectable limit.

For determination of limit of detection of THC, CBD and CBN, 0.5–10 µl of standard samples were spotted on three different TLC plates and plates were developed in toluene (solvent system 1). The average R_f values of the standard samples are calculated and is presented in Table 1.

Ganja and Charas were extracted both in acetone and methanol. The TLC's were performed with both acetone and methanol extracts alongwith standard sample of THC, CBD and CBN in solvent system 1, 2, 3 (given before) for selection of suitable solvent system for separation of cannabinoids. R_f values of the standard samples and RR_f i.e. R_f value with respect to R_f value of CBD are calculated and presented in Table 2. Acetone extracts appeared to be satisfactory as most of the charas and ganja samples were found to dissolve in acetone. Results on comparative evaluation of solvent system 2, 4, 5 are presented in Table 3. Results for determination of the best developing solvent system for opium alkaloids and their derivatives and that for cocaine, methaqualone are presented in Tables 4 and 5.

Table 2. R_f values of CBD, THC, CBN for selection of suitable solvent systems for separation of cannabinoids

Solvent system 1 :	CBD	THC		CBN	
	R_f	R_f	RR_f'	R_f	RR_f'
Reference	0.59	0.52	0.88	0.53	0.89
Sample-1 in acetone	0.59	0.53	0.89	0.53	0.89
Sample-1 in methanol	0.60	0.53	0.88	0.53	0.88
Sample-2 in acetone	0.62	0.54	0.87	0.54	0.87
Sample-2 in methanol	0.62	0.54	0.87	0.54	0.87
Solvent system 2 :					
Reference	0.66	0.54	0.82	0.52	0.79
Sample-1 in acetone	0.65	0.59	0.91	0.53	0.81
Sample-1 in methanol	0.65	0.59	0.91	0.53	0.81
Sample-2 in acetone	0.63	0.56	0.88	0.52	0.82
Sample-2 in methanol	0.64	0.57	0.89	0.53	0.83
Solvent system 3 :					
Reference	0.84	0.79	1.06	0.80	0.95
Sample-1 in acetone	ND			0.79	0.94
Sample-1 in methanol	ND			0.79	0.94
Sample-2 in acetone	ND				
Sample-2 in methanol	ND	0.84	-	0.79	0.94
	ND	0.84	-	0.79	0.94

1 = ganja, 2 = charas.

RR_f' = relative to CBD i.e. R_f of THC/R_f of CBD and R_f of CBN/R_f of CBD respectively.

Gas liquid chromatographic experiments were performed using Hewlet Packard 5890 series II GC with the following parameters :

Column temperature 240 °C for cannabis products
250 °C for opium alkaloids
220 °C for cocoa products

Injector/detector/temperature 280 °C in all cases. Carrier gas nitrogen, flow rate 40 ml/min, column glass SE 30 (3%) for cannabis and cocoa products but for opium alkaloids, column glass 3%, OV-17, length 2 m, id 2 mm in all cases.

The results of the experiments are given in Tables 6–8.

Table 3. R_f values of CBD, THC, CBN for comparative evaluation of developing solvent systems

Solvent system 2 :	CBD	THC		CBN	
	R_f	R_f	RR_f'	R_f	RR_f'
Reference	0.66	0.54	0.82	0.52	0.79
S_1	0.65	0.59	0.90	0.53	0.81
S_2	0.63	0.56	0.88	0.52	0.8.1
Solvent system 4 :					
Reference	0.29	0.23	0.79	0.20	0.69
S_1	0.25	0.29	1.16	0.19	0.76
S_2	0.25	0.30	1.20	0.20	0.80
Solvent system 5 :					
Reference	0.70	0.50	0.71	0.72	1.02
S_1	0.68	0.41	0.60	0.64	0.94
S_2	0.62	0.42	0.67	0.65	1.04

RR_f = Relative to CBD, S_1 = ganja, S_2 = charas.

Table 4. R_f values of different constituents of opiates for determination of the satisfactory developing solvent system

Sample	Solvent system 6		Solvent system 7		Solvent system 8	
	R_f	RR_f'	R_f	RR_f'	R_f	RR_f'
Morphine base	0.37	0.86	0.06	0.17	0.44	1.00
Morphine.HCl	0.37	0.86	0.05	0.14	0.44	1.00
Codeine base	0.43	1.00	0.35	1.00	0.44	1.00
Thebaine base	0.72	1.42	0.77	2.2	0.57	1.29
Papaverine base	0.87	2.02	ND		0.51	1.16
Narcotine base	ND		0.78	2.22	ND	
Opium extract	0.34	0.79	0.05	0.16	ND	
S_1	0.33	0.76	0.06	0.17	0.48	1.09
S_2	0.33	0.76	0.07	1.62	0.48	1.09
Heroin	0.72	1.67	0.56	1.62	0.59	1.34
S_3	0.78	1.81	0.58	1.66	0.56	1.27

RR_f Relative to codeine.

Table 5. R_f values of cocaine and methaqualone

Sample	Solvent system 9		Solvent system 10		Solvent system 11	
	R_f	RR_f	R_f	RR_f	R_f	RR_f
Cocaine	0.80	1.00	0.58	1.00	0.80	1.00
Methaqualone	0.86	1.075	0.48	0.82	0.89	1.11
S_1	0.81	1.01	0.60	1.03	0.76	0.95
S_2	0.86	1.075	0.46	0.79	0.88	1.10

RR_f Relative to cocaine.

Results and discussion

The results of the study are presented in Tables 1-8. It has already been pointed out that one of the important task of forensic chemist to analyze the illicit narcotic analgesic and addictive drugs like cannabis, heroine, cocoa and related products. Naturally, for the analysis of the illicit samples, TLC methods with suitable solvent systems are used. It is necessary to find out suitable solvent systems for TLC analysis i.e. for screening and identification of drugs and drugs with adulterants or diluents and the R_f values in these systems.

Table 6. Retention time in minutes

	CBD		THC		CBN	
	R_t	RR_t	R_t	RR_t	R_t	RR_t
Reference	3.79	1.00	4.47	1.18	5.07	1.34
S_1 (Methanol)	3.81	1.00	4.49	1.17	5.14	1.35
S_2 (Acetone)	3.82	1.00	4.49	1.17	5.15	1.35
S_2 (Acetone)	3.85	1.00	-	-	5.10	1.33

RR_f Relative to CBD.

This necessarily demands the searching and standardization of drugs in suitable solvent systems so that analysis can be done more efficiently. Moreover, the results can be utilized to prepare a database for chromatography to be utilized universally. From the comparision of the database from different laboratories it is possible to devise ways and means for further improvements of methods with elimination of draw backs if any, in the existing system. The results from the Table 1 show that detectable limits is 1 μg for CBD, 0.5 μg for THC and CBN.

Table 7. Retention time of different drugs in minutes

Drugs	Retention time	RR_t
Morphine base	5.406	1.082
Codeine base	4.995	1.000
Thebaine base	5.965	1.194
Papaverine base	9.040	1.81

RR_t = Relative to codeine.

Table 8. Retention time of different drugs in minutes

Substance	Retention time	RR_t
Cocaine	6.240	1.00
S-1	6.215	0.99
Methaqualone	3.435	
S-2	3.284	

RR_f = Relative to cocaine.

From Table 2 - Solvent system 2 (petroleum ether, diethyl ether 80 : 20 appears to be the best TLC solvents system due to following reasons -

(i) separation of the analyte was distinct and satisfactory,

(ii) R_f values of different constituents differ appreciably to be identified individually and properly,

(iii) diethyl ether is more volatile than toluene and petroleum ether so that the spots can be visualized quickly and easily after development.

Developing solvent system numbers 2, 4, 5 for cannabinoids appeared to be satisfactory. In case of opium alkaloids, salt or base appeared to have no effect on TLC but in the solvent system 6 and 8 separation was satisfactory and 6 was considered to be the best developing solvent system. The movement of the solute/solvent was very slow for solvent system 7 and visualization under UV light (254 nm) could not be achieved after development due to low volatility of cyclohexane. Solvent system 10 happen to be the best for cocoa product and for methaqualone and separation was satisfactory.

Retention times of different drugs obtained from gas liquid chromatography experiment were presented in Tables 6-8. The results suggest that the qualitative and quantitative (with reference to standards) analysis of these drugs can easily be carried out using gas liquid chromatography without any interference.

Absence of THC and low content in CBD can be ascribed to the conversion of THC and part of CBD to CBN due to old age of the cannabis plant.

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