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Profiling

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ORIGINAL ARTICLE

Chemical profiling of ecstasy recovered from around Jakarta by High Performance Thin Layer Chromatography (HPTLC)-densitometry

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KEYWORDS

Ecstasy tablet;
Drugs profiling;
HPTLC-densitometry

Abstract In the current study, we identify the use of HPTLC-densitometry and cluster analysis of major substances in profiling seized ecstasy tablets from around Jakarta. One hundred milligrams of a homogenized drug sample was dissolved in 5 ml of pH 10.5 phosphate buffer solution and extracted with 1 ml toluene. The two micro litter of extract spotted on two HPTLC Si GF 254 (20 × 10 cm) plates, then eluted on twin chamber with TB (cyclohexane:toluene:diethylamine 75 + 15 + 5 v/v) and TAEA (toluene:acetone-ethanol:conc.ammonia, 45 + 45 + 7 + 3 v/v) separately. The spots were scanned by TLC-Scanner 3 Camag at 210 nm. The UV-in situ spectrum of each peaks was scanned at 190–400 nm. Corrected hRf-value (hRfc) and insitu spectrum of chromatogram were used to confirm the identity of unknown drugs. Clustering of chromatograms of extracted ecstasy samples was based on their hRfc and AUC of peak. This method was best implemented for street drug identification and grouping a sample into a cluster based on their chemical characteristic.

1. Introduction

Southeast Asia sub-region has suffered from problems of production, trafficking and abuse of tablet ecstasy over the past 20 years. The authorities seized large amounts of amphetamine derivate tablets that were smuggled to Indonesia or to other countries.

Drugs profiling analysis identifies precursors, intermediates, impurities, and reaction by-products that provide useful information regarding the synthetic route and origin of the drug. Between bath product ecstasy tablets contain different amounts of MDMA and impurity profile. This chemical profile can be used as finger-print, which as signatures in propose to establish a link between samples or otherwise. A statistical approach has been used to compare each data by interpreting chemical links between samples [1–4].

HPTLC obtains high resolution and reproducibility in discriminating substances, does a batch analysis and reduces analytical cost. The association of densitometric measures permits to obtain the chromatogram of a sample and their in situ UV-spectra of each detected peaks of its chromatogram. Chemometric software possibly compares between in situ UV

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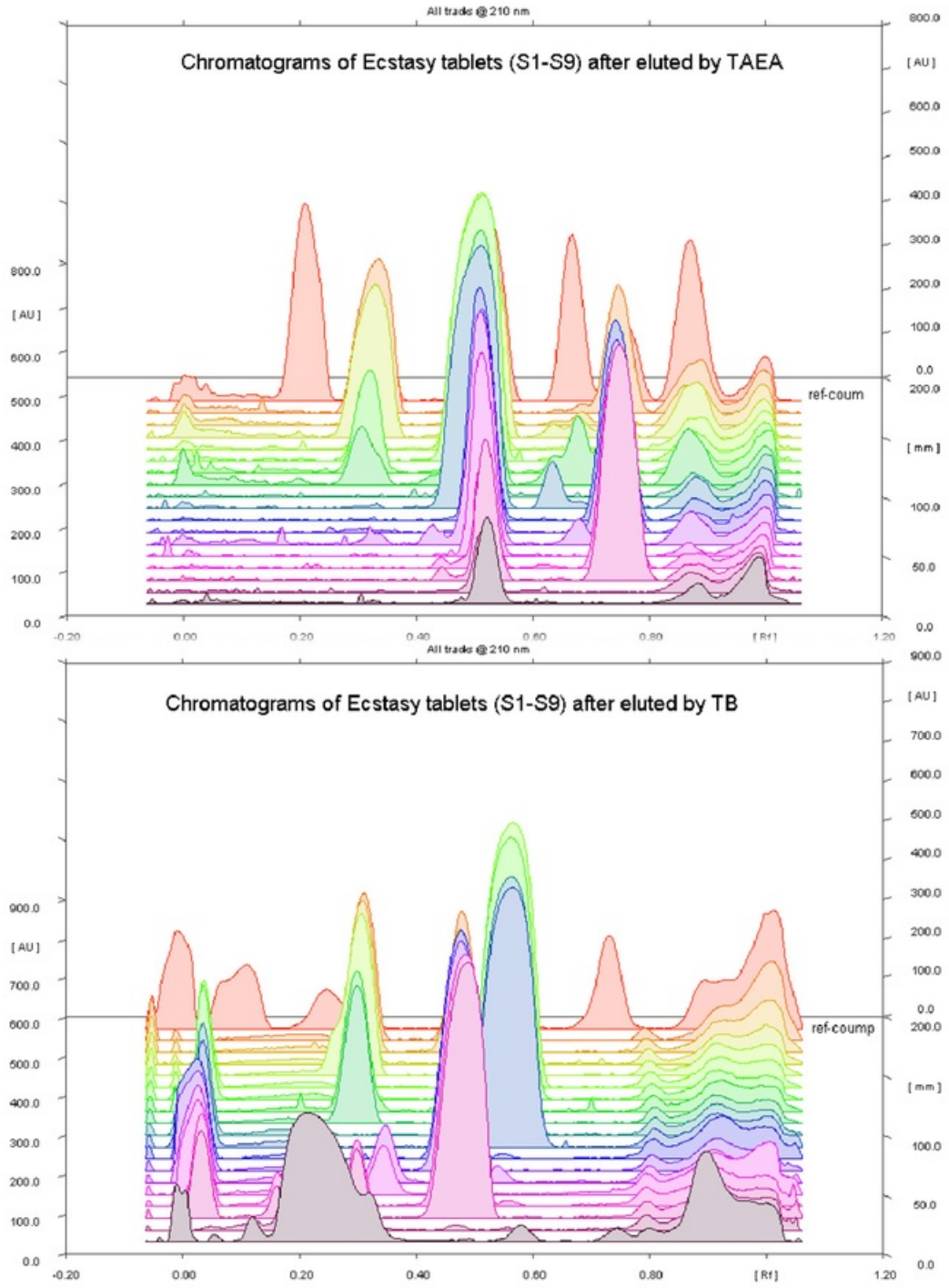

Figure 1 Densitogram of extracted ecstasy tablets (S 1–S 9) after eluted by TAEA and TB, scanned on wavelength 210 nm.

spectrum-data and the library in order to identify substances contained in an ecstasy tablet.

The objectives of this study are to develop the use of HPTLC-densitometric for chemical identification and to

Table 1 The hRf-value of reference compounds within difference plates after eluted on two systems.

Reference compounds	hRfc Ref.	hRf found		
		plat1	plat2	plat3
System TAEA				
Theophylline	16	21	33	34
Caffeine	48	52	63	62
Papaverine	55	67	77	76
Bromhexine	83	87	99	97
System TB				
Theophylline	1	0	0	1
Papaverine	8	11	7	14
Dextromethorphan	42	57	51	73
Amitriptyline	50	73	68	93
Bromhexine	69	89	87	100

characterize on drug profiling. Based on HPTLC-densitometry, we identified of street drugs constituent ecstasy tablets seized around Jakarta and clustered ecstasy tablets.

2. Experimental

2.1. Chemicals and materials

Chemicals (cyclohexane, toluene, diethylamine, ethanol, methanol, acetone, conc. ammonia, potassium dihydrogenphosphate, potassium hydroxide) were of analytical-grade from Merck-Germany, HPTLC silica 60G F_{254} (20 × 10 cm) was also from Merck-Germany. Internal reference compounds to correct the observed Rf-values (morphine, theophylline, caffeine, bromhexine, papaverine, dextromethorphan, and amitriptyline) were obtained from Indonesia Food and Drugs Supervisory Agency-Jakarta. The seized ecstasy tablets were obtained from Indonesian National Narcotics Agency-Jakarta. The concentration of each reference substances was 1 mg/ml in methanol.

2.2. Sample preparation

2.2.1. Profiling of ecstasy tablets

We took 54 of ecstasy tablets in this study. Each tablet was grinded into powder separately, 200 mg of powdered sample was dissolved in 5 ml of 0.2 M phosphate buffer (pH 10.5). The suspension was shook at 300 rpm for 30 min, centrifuged and 4 ml supernatant was transferred into centrifuge tube. Extraction was by ultrasonicating with 1 ml of toluene. After centrifugation, 0.5 ml of organic layer was transferred into an effendorf tube.

2.3. HPTLC-densitometry

Before use the plates were washed with methanol, dried in oven 120 °C for 20 min. The activated plates were equilibrated and stored in a desiccators'. The internal standard compounds and the extracted tablet samples were spotted on two plates separately, by the use of NANOMAT IV. The volume plotted was 2 µL by using capillary (Camag-Switzerland). For the first application x = 10 mm, y = 10 mm, the space between tracks was 10 mm, for the extract of ecstasy tablets was used HPTLC 20 × 10 cm, so we had 19 spots, and standard compounds for

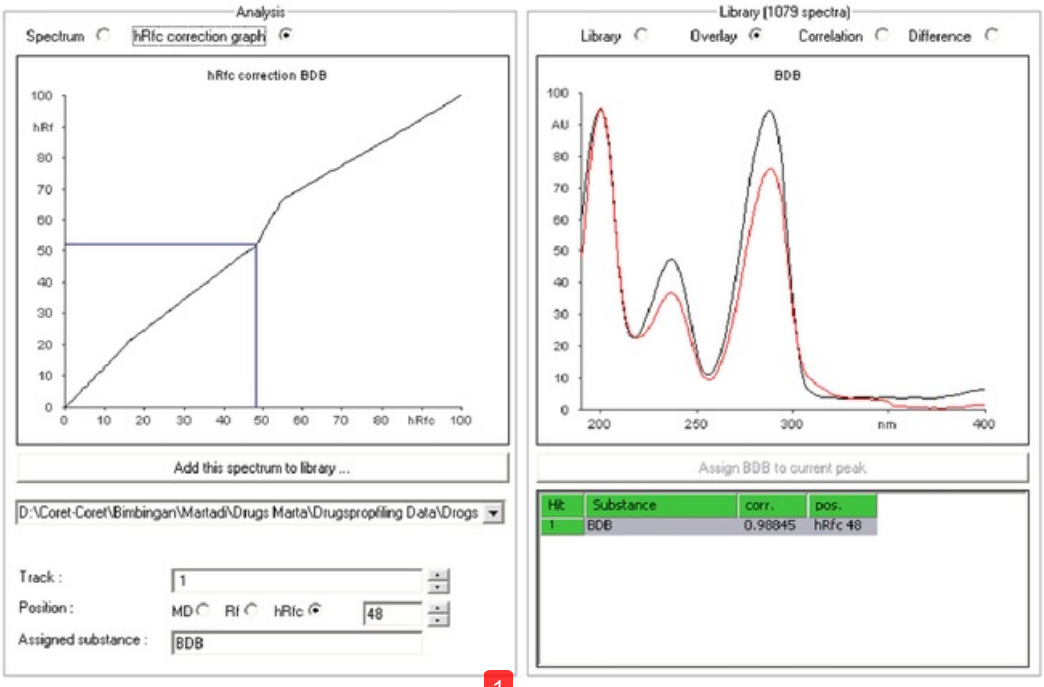

Figure 2 The polygonal method for the correction of hRfc and library search hit list obtained by hRfc + correlation for the spot at hRfc 48 on track 1 (S 1–1) and the best match superimposed with the sample spectrum after eluted by TAEA.

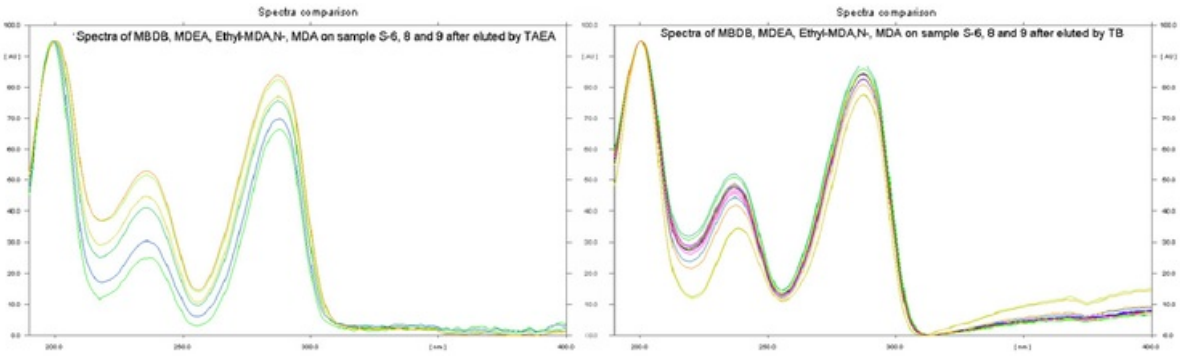

Figure 3 Spectra of detected amphetamines on Samples S-6, 8, and 9, which their spectrum matched as MBDB, MDEA, Ethyl-MDA,N, and MDA.

corrected hRf were spotted on track 19th. Every spotted sample was developed to a distance of 8 cm. One plate was developed on system TB (cyclohexane:toluene:diethylamine 75 + 15 + 5 v/v) and the other on TAEA (toluene:acetone-ethanol:concentrated ammonia, 45 + 45 + 7 + 3 v/v) at room temperature in glass twin-trough chambers (10 cm × 20 cm, with metal lids; Camag, Switzerland) previously saturated with the mobile phase vapor for 30 min. After development, the plates were dried on Camag draying plate at 60 °C for 10 min.

The scanning densitometer was a Camag TLC Scanner 3 operated with WinCATS – Planar Chromatography Manager version 1.4.2.8121 software (Camag, Switzerland). The spots were scanned by absorbance at 210 nm. The scanner was set for maximum light optimization with slit dimension 4.00 × 0.30 mm, scanning speed 20 mm s^{-1}, data resolution 100 µm/step. Spectra of each peaks were recorded in the range of 190–400 nm on all detected peaks' mode, slit dimension 6.00 × 0.30 mm, optimize optical system was resolution, scanning speed 100 nm s^{-1}, data resolution 1 nm/step, reference spectrum x = 10.0 mm, y = 5.0 mm.

2.4. Data processing

The obtained hRfc values were corrected by polygonal method [5] using five reference compounds: for system TB were theophylline (hRfc = 1), papaverine (8), dextromethorphan (42), amitriptyline (50) and bromhexine (69), for system TAEA were morphine (hRfc = 8) theophylline (16), caffeine (48), papaverine (55), and bromhexine (83). To conform the identification of every peaks-chromatograms of extracted ecstasy tablets used WinCATS-Speclib-tool (Camag, Switzerland) by using an hRfc pre-search with a window size of ±5 units of hRfc-found and the correlation-value between in situ spectra and correct library spectra was set minimum on 0.8. The hRfc reference of TB system was obtained from Ref. [5].

For clustering purpose, the HPTLC-densitometry data were transferred to a Microsoft Office Excel 2003 spreadsheet. The area under curve (AUC) of every chromatogram-peak was arranged based on their hRfc. Cluster analysis was carried out using the MINITAB-14 Software. Chromatograms were clustered with multi-variance complete linkage and correlation coefficient distance method.

3. Results and discussion

3.1. Physical and chemical characteristics

The diameters of ecstasy tablets ranged from 7.14 to 9.54 mm, their thickness ranged from 3.82 to 6.13 mm and thier weights ranged from 203.6 to 385.3 mg. Fig. 1 presents the 3D representation of densitogram of extracted ecstasy tablet samples (S 1–S 9). Scanning the spots extracted tablets on wavelength 210 nm showed better densitogram results.

The hRf-value of all reference compounds on two systems varied between plates (see Table 1). This variation is governed by many factors such as the amount of drug applied to the plate, running distance, state of saturation of chamber, etc. The effect of these factors can be reduced by the use of reference compounds and using hRfc [5]. The polygonal method for the correction of hRfc and library search was done automatically by WinCATS-Speclib-tool (see Fig. 2). Fig. 3 shows spectrum of detected amphetamines from extracted ecstasy tables of samples (S-6, 8, and 9). This method was very useful for better indention unknown street drugs from a chromatogram of densitometry.

Tables 2 and 3 show the best library matches and the corresponding spectrum correlations for all spots in 54 extracted ecstasy tablets after they were eluted by TB or TAEA systems, respectively. Library search was based on hRfc of chromatograms. When comparing the best library matches between two systems, we found different chemical identity of a spot between the mobile phases. For example S 1–1 on TB was found two best matches (MDA and BDB) but on TAEA, we finally retrieved BDB. Theoretically MDA and BDB should be separated in these two systems. Undetected MDA on TAEA could be due to minor constituent of sample. BDB was detected on the two systems and can be assumed that BDB as a major component of this sample. On the other hand, separation extracted sample S-6–2 on two systems just gave one peak on every system, but we obtained different best library matches. Observation on library matches (HF, hit factor) on two systems of this sample, we found, that MDEA, MBDB, and Ethyl-MDA, N- were belonged to HF on two systems (see Table 4). These amphetamines have relatively the same hRfc on these systems and have relatively the same spectrum profile, which means

these amphetamines could not be separated and the spectra were not discernible.

Chromatograms of samples S 11–1, S 12–2, S 13, S 17, S 18–1, S 22, and S 27–1 on system TB were presented a peak with relative same hRfc value (20–24). Library searching these peaks were found, that MDMA, DOET, and DMA were fall into hit factor and presented correlation spectrum value more then 0.9000. These amphetamines could be separated into two peaks after eluted by TAEA. The first peak was identified as MDMA within hRfc between 13 and 16, and the second peak was DOET or MDA with hRfc 34–36. Different separation characteristic of the two systems could help to differentiate amphetamines.

Table 4 presents the library matches of each sample, which were recorded on TB as well as TAEA. Identification of unknown drugs based on TLC-densitometry using the two mobile phases can help the narrow selection of library matches into focusing identification. This method can be helpful for small laboratories on drug identification, which do not have GC–MS or LC–MS.

The frequency detected chemical compounds in each ecstasy tablet are shown in Fig. 4. Forty three tablets contained amphethmine derivate and 24 tablets contained MDMA as the sole active ingridient. Ketamine was found in 9 tablets.

Table 2 Grouping of ecstasy tablets and best library matches of 27 pair of samples after eluted by TB.

Group	ID	hRfc found	HF	The best library match	Correl. Value	Group	ID	hRfc found	HF	The best library match	Correl. Value
#1	S 1–1	15	3	MDA	0.96208	#6	S 3–1	2	5	Caffeine	0.87166
		42	1	BDB	0.97897		S 3–2	2	5	Caffeine	0.88024
	S 1–2	16	1	MDA	0.81994	#2	S 20–1	3	6	Theophylline	0.90914
		42	1	BDB	0.88478			42	4	Dextromethorphan	0.97706
#3	S 5–1	2	6	Caffeine	0.89012		S 20–2	3	6	Theophylline	0.88356
		42	3	Dextromethorphan	0.98331			42	3	Dextromethorphan	0.97372
	S 5–2	2	6	Caffeine	0.90418	#7	S 6–1	22	7	MDEA	0.97941
		42	4	Dextromethorphan	0.98292		S 6–2	22	7	MDEA	0.98048
	S 7–1	2	6	Caffeine	0.90486		S 8–1	22	7	MBDB	0.98528
		42	4	Dextromethorphan	0.98293			45	1	BDB	0.80122
	S 7–2	2	6	Caffeine	0.88161		S 8–2	22	7	MDEA	0.98192
		42	4	Dextromethorphan	0.98416			45	1	BDB	0.81022
	S 23–1	2	5	Theophylline	0.93171		S 9–1	23	7	MBDB	0.98354
		35	7	Ketamine	0.98514			35	2	Phenylpropanolamine	0.92744
	S 23–2	2	5	Theophylline	0.93736		S 9–2	23	7	MDEA	0.98195
		35	7	Ketamine	0.99886			35	6	Ketamine	0.90182
	S 25–1	3	7	Theophylline	0.92031		S 10–1	3	7	Caffeine	0.97816
		35	7	Ketamine	0.98849			9	2	Strychnine	0.83889
	S 25–2	2	7	Theophylline	0.91572			24	3	Diazepam	0.96011
		35	7	Ketamine	0.98953		S 11–1	23	8	Ethyl-MDA,N-	0.99673
#4	S 21–1	22	7	MBDB	0.98523			35	8	Ketamine	0.95928
	S 21–2	21	7	MBDB	0.98548		S 11–2	23	8	Ethyl-MDA,N-	0.99661
	S 22–1	21	7	MBDB	0.98081			35	7	Ketamine	0.95986
	S 22–2	21	7	MDEA	0.98196		S 12–1	23	8	Ethyl-MDA,N-	0.99659
	S 24–1	2	5	Theophylline	0.85522		S 12–2	23	8	Ethyl-MDA,N-	0.99676
		21	7	MDEA	0.97585		S 13–1	24	7	Ethyl-MDA,N-	0.99684
		34	6	Phenylpropanolamine	0.93821		S 13–2	23	8	Ethyl-MDA,N-	0.99681
	S 24–2	21	7	MBDB	0.98340		S 14–1	23	8	Ethyl-MDA,N-	0.99683
	S 26–1	21	7	MBDB	0.98329		S 14–2	23	8	Ethyl-MDA,N-	0.99561
	S 26–2	21	7	MBDB	0.98489		S 16–1	23	8	Ethyl-MDA,N-	0.99505
	S 27–1	22	7	MDMA	0.99004		S 16–2	24	7	Ethyl-MDA,N-	0.99578
	S 27–2	22	7	MDMA	0.98932		S 17–1	24	7	Ethyl-MDA,N-	0.99696
	S 28–1	22	7	MDMA	0.98953		S 17–2	24	7	Ethyl-MDA,N-	0.99687
		35	6	Phenylpropanolamine	0.94404	#8	S 10–2	3	7	Theophylline	0.97796
	S 28–2	23	7	MDMA	0.99010			8	3	Strychnine	0.85139
		35	5	Phenylpropanolamine	0.93827			23	3	Diazepam	0.93534
#5	S 2–1	2	3	Caffeine	0.85063		S 18–1	3	7	Theophylline	0.97227
		36	4	Ketamine	0.99599			23	7	MDEA	0.96968
	S 2–2	2	3	Caffeine	0.86546		S 18–2	3	7	Theophylline	0.96877
		36	3	Ketamine	0.99630			23	7	MDEA	0.97244
	S 4–1	2	6	Caffeine	0.88945		S 19–1	3	7	Theophylline	0.97465
		35	6	Ketamine	0.99999			24	6	Ethyl-MDA,N-	0.99333
	S 4–2	2	6	Caffeine	0.88559		S 19–2	3	7	Theophylline	0.97803
		35	6	Ketamine	0.99999			24	7	Ethyl-MDA,N-	0.99689

Abbreviations: HF (hit factors, numbers of library matches on the conditional searching), ID (sample identities), MDMA (3,4-Methylendioxymethamphetamine), MDA (3,4-Methylendioxyamphetamine), B 3,4-methylendioxyphenyl-butanamine), MDEA (3,4-Methylenedioxyethylamphetamine), DOET (2,5-Dimethoxy-4-ethyamphetamine), MBDB (N-Methyl-1-(1,3-benzodioxol-5-yl)-2butanamine).

Table 3 Grouping of ecstasy tablets and best library matches of 27 pair of samples after eluted by TAEA.

Group	ID	hRfc found	HF	The best library match	Correl. Value	Group	ID	hRfc found	HF	The best library match	Correl. Value
#1	S 1–1	48	1	BDB	0.98845	#4	S 12–1	25	3	MDEA	0.89157
	S 1–2	48	1	BDB	0.98133		S 18–1	15	3	MDMA	0.92399
	S 5–1	47	10	Caffeine	0.90944			35	3	DOET	0.84575
	S 5–2	46	14	Isoxsuprine	0.93657			45	8	Caffeine	0.97956
		46	14	Isoxsuprine	0.93657		S 18–2	16	2	MDMA	0.91583
	S 6–1	26	4	MDEA	0.97958			36	9	DOET	0.77429
	S 6–2	27	5	Ethyl-MDA,N-	0.99651			46	8	Caffeine	0.97758
	S 7–1	26	3	Ethyl-MDA,N-	0.97171		S 21–1	15	3	MDMA	0.93616
		48	11	Caffeine	0.92058		S 21–2	15	3	MDMA	0.94401
	S 7–2	26	4	MDEA	0.93304		S 24–1	13	2	MDMA	0.92363
		48	10	Caffeine	0.88790		S 24–2	13	1	MDMA	0.89491
	S 8–1	28	10	Ethyl-MDA,N-	0.98897	#9	S 28–1	17	6	MDMA	0.97473
		47	11	Isoxsuprine	0.92218			69	12	Pipradrol	0.97573
	S 8–2	28	8	Ethyl-MDA.N-	0.99539		S 28–2	18	4	MDMA	0.95696
		46	13	Isoxsuprine	0.93779			69	13	Pipradrol	0.97257
	S 9–1	10	14	Benztropine	0.97945						
		29	13	MDA	0.99223	#6	S 20–1	40	12	Embutramide	0.93255
		46	11	Isoxsuprine	0.93681			47	4	Caffeine	0.98277
		66	12	Ketamine	0.98692		S 20–2	39	11	Norverapamil	0.93889
	S 9–2	10	14	Benztropine	0.97948			47	4	Caffeine	0.98025
		29	13	MDA	0.99228	#7	S 26–1	14	2	MDMA	0.92871
		46	11	Isoxsuprine	0.93688		S 26–2	14	2	MDMA	0.87758
		66	12	Ketamine	0.98694		S 27–1	15	3	MDMA	0.95982
	S 19–1	46	5	Caffeine	0.97455			46	8	Caffeine	0.97301
	S 19–2	47	5	Caffeine	0.97977			69	13	Pipradrol	0.98003
#2	S 23–2	14	1	MDMA	0.92704		S 27–2	16	5	MDMA	0.96941
		15	7	Theophylline	0.96499			71	9	Bupivacaine	0.88894
		33	12	Embutramide	0.91415	#8	S 10–1	15	4	Theophylline	0.92394
		45	12	Caffeine	0.98901			19	2	Isoetarine	0.88670
		54	8	Papaverine	0.94817			38	12	Embutramide	0.92175
		68	13	Pipradrol	0.98563			46	4	Caffeine	0.98029
	S 25–1	45	8	Caffeine	0.98211			54	9	Papaverine	0.94816
		69	13	Pipradrol	0.98157			65	7	Prilocaine	0.86715
	S 25–2	13	2	MDMA	0.92241		S 10–2	45	8	Caffeine	0.97174
		16	1	Theophylline	0.80499			66	7	Diazepam	0.95443
		33	10	Embutramide	0.88246		S 22–1	14	2	MDMA	0.88190
		45	11	Caffeine	0.98979		S 22–2	14	8	MDMA	0.94804
		54	3	Papaverine	0.90043			34	14	Embutramide	0.92001
		68	13	Lidocaine	0.97686			46	8	Caffeine	0.97121
#3	S 2–1	48	4	Caffeine	0.98529		S 23–1	45	10	Caffeine	0.98820
		66	11	Dextromoramide	0.96400			66	11	Ketamine	0.98225
	S 2–2	48	4	Caffeine	0.98069	#10	S 11–1	17	4	MDMA	0.96414
		66	12	Ketamine	0.97628			36	8	Embutramide	0.90968
	S 3–1	47	7	Caffeine	0.99464			45	6	Caffeine	0.96018
	S 3–2	47	7	Caffeine	0.99618			53	5	Papaverine	0.92459
	S 4–1	47	7	Caffeine	0.99587			66	12	Ketamine	0.98493
		66	13	Ketamine	0.98733		S 11–2	25	3	MDEA	0.94048
	S 4–2	47	7	Caffeine	0.99628		S 12–2	15	2	MDMA	0.91784
		65	13	Ketamine	0.98516		S 13–1	15	6	MDMA	0.96670
#5	S 16–1	15	3	MDMA	0.93922			35	12	Embutramide	0.92431
	S 16–2	15	4	MDMA	0.97559			45	14	Caffeine	0.96973
	S 17–1	15	3	MDMA	0.95804		S 13–2	15	4	MDMA	0.96985
		35	2	DOET	0.83412			34	9	Embutramide	0.89282
	S 17–2	16	3	MDMA	0.94801		S 14–1	15	2	MDMA	0.90690
		35	8	Embutramide	0.89929		S 14–2	15	2	MDMA	0.90344

Abbreviations: see Table 2.

3.2. Similarity of the pair of ecstasy tablet and clustering of samples

Drugs profiling is a study to establish a link between one to the other illicit drugs based on their chemical characteristic. A chromatogram can be used as a fingerprint of one sample. Separation properties (hRfc) of their chemical constituent and the AUC of each substance are unique for each sample. The hRfc of a spot chromatogram represented its chemical identity and AUC stood for its concentration.

Table 4 The library matches on chemical identification every spotes chromatogram of extracted ecstasy tablets, which were found after they were eluted by TB as well as TAEA.

No	ID	Library matches on TB as well as TAEA	No	ID	Library matches on TB as well as TAEA
1	S 1–1	BDB	29	S 16–1	MDMA
2	S 1–2	BDB	30	S 16–2	MDMA
3	S 2–1	Caffeine, Ketamine, Lidocaine	31	S 17–1	MDMA, DOET, DMA
4	S 2–2	Caffeine, Ketamine, Lidocaine	32	S 17–2	MDMA, DOET, DMA
5	S 3–1	Caffeine	33	S 18–1	MDMA, DOET, Caffeine
6	S 3–2	Caffeine	34	S 18–2	MDMA, Caffeine
7	S 4–1	Caffeine, Ketamine, Lidocaine	35	S 19–1	Caffeine
8	S 4–2	Caffeine, Ketamine, Lidocaine	36	S 19–2	Caffeine
9	S 5–1	Caffeine, BDB	37	S 20–1	Caffeine
10	S 5–2	Caffeine, BDB, Mianserin, Metixene	38	S 20–2	Caffeine
11	S 6–1	MDEA, MBDB, Ethyl-MDA,N-	39	S 21–1	MDMA
12	S 6–2	MDEA, MBDB, Ethyl-MDA,N-	40	S 21–2	MDMA
13	S 7–1	Caffeine, Dextromethorphan, BDB	41	S 22–1	MDMA
14	S 7–2	Caffeine, Dextromethorphan, BDB	42	S 22–2	MDMA, DOET, DMA
15	S 8–1	MBDB, MDEA, Ethyl-MDA,N-, BDB	43	S 23–1	Ceffeine, Ketamine
16	S 8–2	MBDB, MDEA, Ethyl-MDA,N-, BDB	44	S 23–2	Theophylline, Caffeine, Diprophylline, Lidocaine
17	S 9–1	MBDB, MDEA, Ethyl-MDA,N-, MDA, DOET, DMA, Ketamine	45	S 24–1	MDMA
18	S 9–2	MBDB, MDEA, Ethyl-MDA,N-, MDA, DOET, DMA, Ketamine	46	S 24–2	MDMA
19	S 10–1	Theophylline, Caffeine, Diprophylline, Diazepam	47	S 25–1	Caffeine, Lidocaine
20	S 10–2	Caffeine, Diazepam	48	S 25–2	Theophylline, Caffeine, Lidocaine
21	S 11–1	MDEA, MBDB, Ethyl-MDA,N-	49	S 26–1	MDMA
24	S 12–2	MDEA, MBDB, Ethyl-MDA,N-	50	S 26–2	MDMA
25	S 13–1	MDMA, DOET, DMA	51	S 27–1	MDMA, DOET
26	S 13–2	MDMA, DOET, DMA	52	S 27–2	MDMA
27	S 14–1	MDMA	53	S 28–1	MDMA, Lidocaine
28	S 14–2	MDMA	54	S 28–2	MDMA, Lidocaine

Abbreviations: see Table 2.

Clustering was based on hRfc and AUC of chromatogram of extracted ecstasy samples. Fig. 5 presents the dendrograms of 27 paired ecstasy tablets. The clustering analysis was done with MINITAB-14 Software under multi-variance complete linkage and correlation coefficient distance method. Based on chromatogram samples on system TAEA, we found 10 clusters and there were two pairs of samples (S 12 and 23), which were not grouped into one cluster (see Table 3). The chromatograms on system TB were grouped into 8 clusters and we found only a pair of S 10 did not come into the group (see Table 2). These pair samples showed different chromatogram between pairs, so we assumed that the pair samples were not from same bath production. The separation power on amphetamines on these systems and the concentration of chemical compound of a tablet produced different chromatograms profile of a sample, these governed different cluster-group members.

Grouping was based on similar chromatogram properties of a sample; it means a similarity of hRfc and AUC of all peaks within a chromatogram. The similarity of chromatograms extracted ecstasy samples within a group cluster can be seen in Tables 2 and 3. It shows that one group is arranged from samples, which had relatively the same chemical characteristic.

Clustering based on chromatograms on system TB resulted in better correlation to their amphetamine ingredients, then clustering was based on system TAEA. Grouping based on chromatograms on system TB could also classify the samples into sub cluster according to their chemical disparity (see Fig. 4).

Nowadays the use of GC–MS or LC–MS on drugs profiling analysis is known worldwide [6,7]. Separation power of these instruments could provide the best separation and identify the substance precisely, this gave better drug profiling analysis.

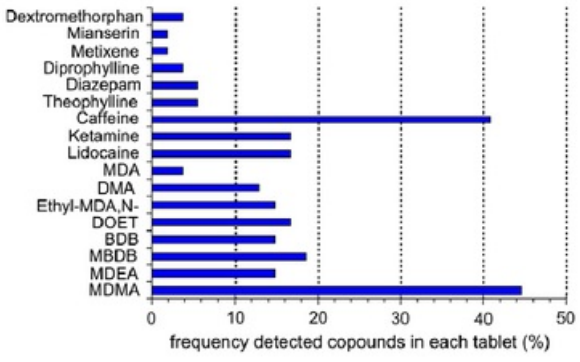

Figure 4 Frequency of chemical compounds detection in each ecstasy tablet.

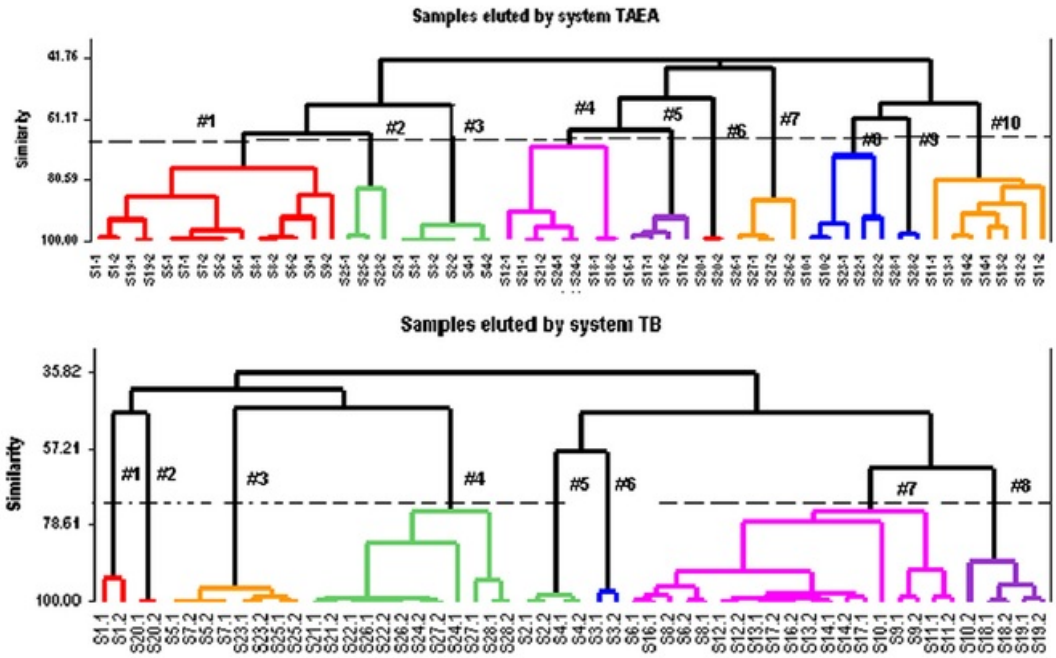

Figure 5 Dendrograms of 27 pair's ecstasy tablets using all chromatogram peaks after eluted in system TAEA and TB with complete linkage and correlation coefficient distance.

The poor separation power of HPTLC compared with GC/LC–MS, was not resulted at high precision identification and profiling analysis. This technique provides a lower analytical cost than GC or HPLC for drug profiling and it consists of an example of how TLC should not be neglected in drugs profiling analysis.

4. Conclusion

The HPTLC method presented in this study successfully identified chemical characterization and clustered the ecstasy tablets based on their chromatograms. This technique also provided a lower analytical cost than GC or HPLC in drug profiling and it showed an example of how TLC should not be neglected in drugs profiling method.

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Acknowledgments

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