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Forensic Analysis of Toners by Thin Layer Chromatography and High Performance Thin Layer Chromatography

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Abstract

The counterfeiting of printed documents is most common phenomenon in the modern world. The aim of the present research is to examine suspected documents from their ink components. 28 raw and processed toners were analyzed for their colored ink components by thin layer chromatography and high performance thin layer chromatography. Chloroform was efficient to extract all dye components from the inks. The separation was performed using two separate mobile phases. It is possible to differentiate toner cartridges by TLC and HPTLC.

Keywords: Questioned documents; Forensic analysis of ink; Toner; TLC; HPTLC

Introduction

The chemical examination of toners helps to determine whether similar or different inks are used to produce the whole document, to establish whether a document could have been produced on the purported date; or to determine the origin of a document [1]. The above questions can be answered by differentiating two toners formulations. Ink formula is a precise recipe or set of ingredients and their quantities that the manufacturer specifies for the final toners product. These ingredients are colorants (dyes and pigments) and vehicle components (volatile solvents, resins, etc.) [2].

The present study deals with the qualitative analysis of colored components of toners by thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC). TLC method is successfully used for the comparison of the toner but cannot be used as an identification method unless used for comparison with a complete collection of standards. HPTLC has been reported to be an automated form of TLC and has an inbuilt mechanism to store the database of analyzed toners samples which can be used for the comparison and identification.

Much of the work has been reported on the analysis of colored component of toner toners by thin layer chromatography [3-5] but limited work has been carried out to recognize the discrimination potential of high performance thin layer chromatography to analyze toners [6-8]. By keeping the above facts in minds, the present study is aimed to determine the discriminating potential of thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) in order to differentiate the colored components of the toner toners samples.

Experimental

In the present study, an attempt has been made to classify the toners on the basis of the colorants (dyes and pigments) by using thin layer chromatography (TLC) and high performance thin layer

Table 1: Toner samples analyzed.

Sample No	Model of Machine	Type of Toner	
1	Konica Minolta Bizhub c450	Raw	Processed
2	Konica Minolta Bizhub c450	Raw	Processed
3	Konica Minolta Bizhub c220	Raw	Processed
4	XEROX 252 PRO C751 EX	Raw	Processed
5	Konica Minolta Bizhub c220	Raw	Processed
6	Konica Minolta Bizhub c224e	Raw	Processed
7	Konica Minolta Bizhub c654	Raw	Processed
8	Xerox 7425	Raw	Processed
9	Konica Minolta Bizhub 6000	-------	Processed
10	Konica Minolta Cannon oce-231	---------	Processed
11	Xerox250	----------	Processed
12	Konica Minolta Bizhub 6500	---------	Processed
13	Konica Minolta Bizhub 6501	Raw	Processed
14	Konica Minolta Bizhub c280	Raw	Processed
15	Xerox7245	------	Processed
16	Konica Minolta Bizhub c450	------	Processed
17	Doculour-12	-------	Processed
18	Universal colour	Raw	----------
19	Doculour-12	Raw	----------
20	Doculour-12	Raw	----------
21	Doculour-12	Raw	----------
22	Xerox 50	Raw	----------
23	Xerox LDH	Raw	----------
24	HP Laserjet 1500L	Raw	----------
25	Xerox DC-12	Raw	----------
26	Canon CLC 300	Raw	----------
27	Universal colour	Raw	----------
28	Doculour-12	Raw	----------

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Table 2: Solvent systems used.

Solvent System Codes	Solvent System	Ratio
I	Etthyl acetate : Ethanol : Distilled water	70:35:30
II	Chloroform : Methanol : n-Hexane : Acetic acid	85:25:5:1
III	n-Butanol :Ethanol :Distilled water	90:15:20
IV	Dichloroethane : Acetone	12:24
V	Carbon Tetra Chloride : Ethyl Acetate	90:33
VI	N-Butanol : Propanol : Acetic acid	60:15:5
VII	N-Butanol : Ethanol : Distilled water : Acetic acid	70:35:30:5
VIII	N-Butanol : Acetone : Distilled water	40:50:10
IX	Etthyl acetate : Ethanol : Chloroform	2:2:10
X	Isoamyl Alcohol : Acetone : Distilled water : Ammonia	15:15:5:1

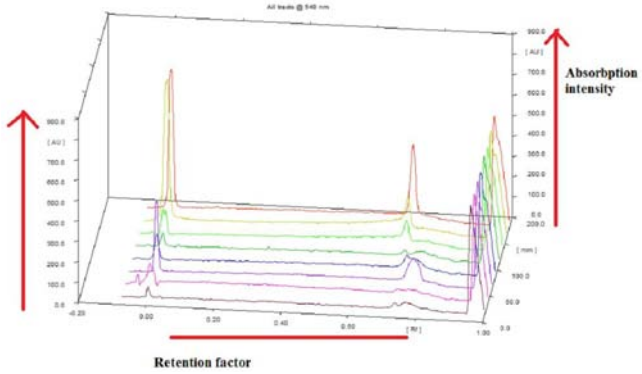

Figure 1: HPTLC 3D spectra of processed toners (sample number 11-14 and 17) and raw toners (sample number 9-11) with Solvent System I.

chromatography (HPTLC).

Collection of sample

Twenty eight raw as well as processed toner samples have been collected and were marked serially (Table 1). The raw toners were taken directly from the ink cartridge whereas a text was designed for the collection of processed toner sample. The text comprised of paragraphs in four colors, that is, cyan, magenta, yellow and black. The designed text was printed on A4 size sheets from different models of laser jet printers/photocopiers.

Sample preparation

The sample preparation of raw and processed toner samples were done separately and marked serially. In raw toner samples, 0.5 micrograms of each of four colors, that is, cyan, magenta, yellow and black were added into a test tube and mixed using 3 ml chloroform. In processed toner samples, total eight alphabets of four colors (two alphabets from each color) were selected from each printed document. Modified *Munson's technique* was used (Tandon *et al.* 1995) to remove toners from the processed toner samples. An aluminum foil was placed on the selected alphabets and the heat was applied on the back of sheet with a soldering rod (30 W) for 1-2 minutes. 2-3 lifts were sufficient to remove an adequate amount of sample. In another test tube, extracts of all four colors of each ink sample were mixed together using 2 ml chloroform. This was done because when a colored image (or printed stroke) was observed microscopically, all four colors visualized as series of dots. For example, if yellow color is examined under the microscope, it is visualized as multiple dots of cyan, magenta, yellow and black color dots. However, they are not of equal intensity on substrate, but intensity of colors does not affect the results. Thus samples are considered as mixture of all colors. Also, the four colors, that is, cyan, magenta, yellow and black were used for printing the picture in layers. Hence, 4-colors were analyzed after mixing [9-10]. The similar process was done for the preparation of all the colored toners (raw and processed). The prepared mixture extracts were analyzed using thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC).

Solvent and stationery phase

The solvents used have been purchased from Loba Chemie Pvt. Ltd., Laboratory Reagent, Mumbai, India while the stationery phase from Merck, Germany. Ten different solvent systems (Table 2) were tried, of which two solvent systems (code I and II) were found to be efficient to separate all the toner samples. Both the solvent systems were kept in separate glass chambers for saturation for 35 minutes at

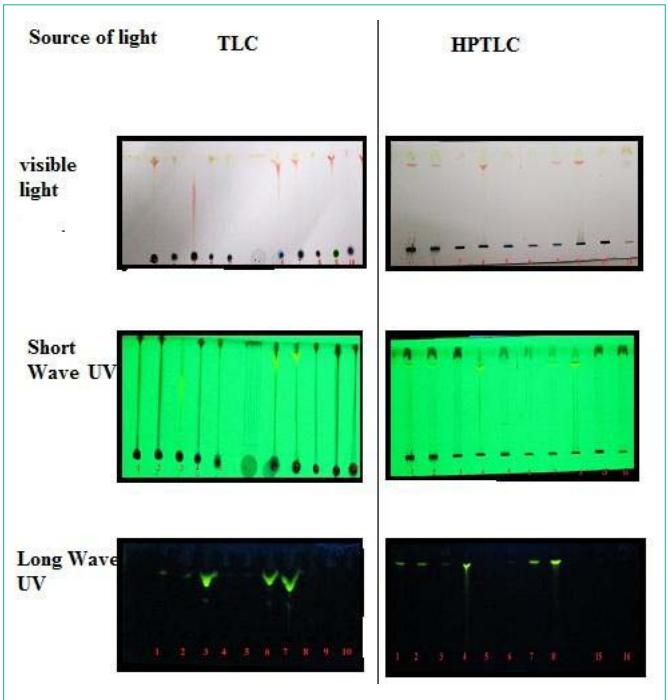

Figure 2: Differentiation of toner samples by TLC and HPTLC with solvent system I and II.

Table 3: Thin Layer Chromatography of toners.

Sample No.	Solvent system (code)	TLC Result with Solvent System I And II (1-28)								
		UNDER DAY LIGHT			UNDER SHORT UV LIGHT			Under long UV light		
		No of Spots	Color of spots	hR_f	No of Spots	Color of Spots	hR_f	No of Spots	Color of Spots	hR_f
1	I	1 1	Yellow Magenta	91.1 88.0	- 1	- Yellow	- 88.0	- 1	- Green	- 88.0
	II	1	Yellow	84.0	-	-	-	-	-	-
2	I	1	Magenta	93.3	-	-	-	-	-	-
	II	1	Magenta	78.0	-	-	-	-	-	-
3	I	1 1	Yellow Magenta	84.4 48.8	1	Yellow	48.8	1	Green	48.8
	II	1 1	Yellow Orange	78.0 54.0	1	Yellow	54.0	1	Green	54.0
4	I	1	Magenta	93.3	-	-	-	-	-	-
	II	1	Magenta	80.0	-	-	-	-	-	-
5	I	1	Yellow	93.3	-	-	-	-	-	-
	II	1	Yellow	80.0	-	-	-	-	-	-
6	I	1 1	Yellow Magenta	93.3 84.4	- 1	- Yellow	- 84.4	- 1	- Green	- 84.4
	II	1 1	Yellow Orange	78.0 58.0	- 1	- Yellow	- 58.0	- 1	- Green	- 58.0
7	I	1 1	Yellow Magenta	95.5 88.0	- 1	- Yellow	- 88.0	- 1	- Green	- 88.0
	II	1 1	Yellow Orange	76.0 52.0	- 1	- Yellow	- 52.0	- 1	- Green	- 52.0
8	I	1	Yellow	93.3	-	-	-	-	-	-
	II	1	Yellow	80.0	-	-	-	-	-	-
9	I	1 1	Yellow Orange	88.0 77.0	- 1	- Yellow	- 77.0	- 1	- Green	- 77.0
	II	1 1	Yellow Orange	90.0 92.0	- 1	- Yellow	- 92.0	- 1	- Green	- 92.0
10	I	1 1	Yellow Orange	86.6 66.6	1 -	Yellow -	66.6 -	1 -	Green -	66.6 -
	II	1 1	Yellow Orange	82.0 54.0	- 1	- Yellow	- 54.0	- 1	- Green	- 54.0
11	I	1	Yellow	88.0	-	-	-	-	-	-
	II	1	Yellow	82.0	-	-	-	-	-	-
12	I	1 1	Yellow Orange	86.6 66.6	- 1	- Yellow	- 66.6	- 1	- Green	- 66.6
	II	1 1	Yellow Orange	84.0 54.0	- 1	- Yellow	- 54.0	- 1	- Green	- 54.0
13	I	1 1	Yellow Magenta	93.3 91.1	-	-	-	-	-	-
	II	1	Yellow	78.0	-	-	-	-	-	-
14	I	1	Pink	95.5	-	-	-	-	-	-
	II	1	Pink	82.0	-	-	-	-	-	-
15	I	1	Magenta	88.0	-	-	-	-	-	-
	II	1	Magenta	86.0	-	-	-	-	-	-
16	I	1 1	Yellow Orange	91.1 71.1	- 1	- Yellow	- 71.1	- 1	- Green	- 71.1
	II	1 1	Yellow Orange	84.0 56.0	- 1	- Yellow	- 56.0	- 1	- Green	- 56.0
17	I	1	Magenta	91.0	-	-	-	-	-	-
	II	1	Magenta	88.0	-	-	-	-	-	-
18	I	1 1	Yellow Magenta	91.1 88.0	-	-	-	-	-	-
	II	1	Yellow	78.0	-	-	-	-	-	-
19	I	1 1	Yellow Magenta	86.0 73.3	-	-	-	-	-	-
	II	1 1	Yellow Magenta	81.2 14.6	-	-	-	-	-	-

20	I	1 1	Yellow Magenta	86.0 73.3	-	-	-	-	-	-
	II	1 1	Yellow Magenta	81.2 14.6	-	-	-	-	-	-
21	I	1 1	Yellow Magenta	86.0 73.3	-	-	-	-	-	-
	II	1 1	Yellow Magenta	79.0 17.7	-	-	-	-	-	-
22	I	1 1 1	Yellow Magenta Blue	91.1 75.0 91.1	-	-	-	-	-	-
	II	1 1 1	Yellow Magenta Blue	76.8 14.6 65.0	-	-	-	-	-	-
23	I	1 1	Yellow Magenta	88.0 75.0	-	-	-	-	-	-
	II	1 1	Yellow Magenta	79.0 17.7	–	–	–	–	–	–
24	I	1 1	Yellow Pink	88.0 66.6	1 -	Yellow -	66.6 -	1 -	Green -	66.6 -
	II	1 1 1	Yellow Pink Blue	79.0 54.0 75.0	1	Yellow	54.0	1	Green	54.0
25	I	1	Yellow	91.1	-	-	-	-	-	-
	II	1	Yellow	79.0						
26	I	1	Magenta	86.0	-	-	-	-	-	-
	II	1	Magenta	80.0						
27	I	1 1	Yellow Pink	88.0 68.8	1	Yellow	68.8	1	Green	68.8
	II	1 1 1	Yellow Pink Blue	79.0 59.0 77.0	1	Yellow	59.0	1	Green	59.0
28	I	1 1	Yellow Magenta	91.1 77.7	-	-	-	-	-	-
	II	1 1	Yellow Magenta	79.0 19.8						

normal room temperature. The precoated silica gel G-60 F254 non fluorescent aluminum plates (6.5 x 20 cm) were used as the stationery phase.

Thin Layer Chromatography

The toner sample was spotted on precoated silica gel non-fluorescent aluminum plate 60 F254 (6.5 x 20 cm) with the help of fine capillary tube. Separate capillary tube was used for each sample. The spotted plate was kept for drying at normal room temperature for 15-20 minutes.

The dried spotted plate was placed gently in the saturated solvent chamber and was allowed to move vertically till it reached the 5 cm mark on the plate.

High Performance Thin Layer Chromatography

Instrumentation

HPTLC instrument consists of CAMAG (Muttenz, Switzerland) Linomat V sample applicator with 100 µl applicator syringe (Hamilton, Switzerland). Chromatography was performed on 6.5 cm × 20 cm non fluorescent aluminum TLC plates precoated with silica gel 60- F254 (E. Merck, Darmstadt, Germany). CAMAG TLC scanner 4 was used for scanning the plates.

Sample application

The 3 µl of each prepared sample was applied on the plate in the form of narrow bands of 6 mm length. The samples were applied under a continuous drying stream of nitrogen gas. The bands were applied 15 mm above from the bottom and 15 mm away from left edge of the plate.

Mobile phase and development

Plates were developed using two separate mobile phases consisting of ethyl acetate: ethanol: distilled water (70:35:30) and chloroform: methanol: n-hexane: acetic acid (85:25:5:1) respectively. Linear ascending development was carried out in a twin-trough glass chambers equilibrated with ten milliliters of the mobile phase. The mobile phase was allowed to migrate at a distance of 50 mm on plates. After development, the plates were dried completely. A similar process was carried out for all the toner samples.

Densitometric analysis

The densitometry scanning was performed with CAMAG TLC scanner 4 in the absorbance-reflectance mode under control by win CATS planar chromatography software (CAMAG, Switzerland). The source of radiation was the deuterium lamp, and bands were scanned at 540 nm. The samples showing absorbance at respective wavelength were recorded in the software in the form of 3D graphs (Figure 1).

Visualization of spots

The developed chromatograms were visualized under visible and ultraviolet light (short and long wavelength) and are shown in Figure

Table 4: High Performance Thin Layer Chromatography of toners.

Sample No.	Solvent system (code)	HPTLC (1-28)								
		Under Day Light			Under Short UV Light			Under Long UV Light		
		No of Spots	Color of spots	hR_f	No of Spots	Color of Spots	hR_f	No of Spots	Color of Spots	hR_f
1	I	1 1 1	Yellow Orange Magenta	90.6 79.2 81.1	1	Yellow	79.2	1	Green	79.2
	II	1	Yellow	78.2	1	Yellow	78.2	1	Green	78.2
2	I	1	Magenta	88.7	-	-	-	1	Green	88.7
	II	1	Magenta	74.5				1	Green	74.5
3	I	1 1 1	Yellow Orange Magenta	86.8 75.4 77.4	1	Yellow	75.4	1	Green	75.4
	II	1	Yellow	76.4	1	Yellow	76.4	1	Green	76.4
4	I	1	Yellow	88.7	-	-	-	-	-	-
	II	1	Yellow	76.4						
5	I	1	Yellow	90.6	-	-	-	1	Green	90.6
	II	1	Yellow	74.5				1	Green	74.5
6	I	1 1 1	Yellow Orange Magenta	88.7 77.4 78.4	1	Yellow	77.4	1	Green	77.4
	II	1 1	Yellow Orange	78.2 63.3	1	Yellow	78.2	1	Green	78.2
7	I	1 1 1	Yellow Orange Magenta	88.7 77.4 78.4	1	Yellow	77.4	1	Green	77.4
	II	1 1	Yellow Orange	80.0 63.4	1	Yellow	80.0	1	Green	80.0
8	I	1	Yellow	90.6	-	-	-	-	-	-
	II	1 1	Yellow Orange	78.2 63.3	-	-	-	-	-	-
9	I	-	-	-	-	-	-	-	-	-
	II	1	Yellow	96.0	1	Yellow	96.0	1	Green	96.0
10	I	-	-	-	-	-	-	-	-	-
	II	1	Yellow	95.0	1	Yellow	95.0	1	Green	95.0
11	I	1 1	Yellow Orange	90.9 85.5	1	Yellow	90.9	1	Green	90.9
	II	1	Yellow	88.0	1	Yellow	88.0	1	Green	88.0
12	I	1 1	Yellow Orange	90.9 85.5	1	Yellow	90.9	1	Green	90.9
	II	1	Yellow	96.0	1	Yellow	96.0	1	Green	96.0
13	I	1 1 1	Yellow Orange Magenta	89.1 83.6 85.5	1	Yellow	83.6	1	Green	83.6
	II	1 1	Yellow Orange	74.2 60.0	1	Yellow	74.2	1	Green	74.2
14	I	1 1 1	Yellow Orange Magenta	89.1 83.6 85.5	1	Yellow	83.6	1	Green	83.6
	II	1 1	Yellow Orange	76.4 63.3	1	Yellow	76.4	1	Green	76.4
15	I	1	Yellow	90.9	1	Yellow	90.9	1	Green	90.9
	II	1	Yellow	98.0	-	-	-	1	Green	98.0
16	I	1	Magenta	88.7	1	Yellow	88.7	1	Green	88.7
	II	1	Yellow	96.0	1	Yellow	96.0	1	Green	96.0
17	I	1 1	Yellow Orange	89.1 83.6	1	Yellow	83.6	1	Green	83.6
	II	1	Yellow	96.0	1	Yellow	96.0	1	Green	96.0

18	I	1 1	Yellow Orange	89.2 83.6	-	-	-	-	-	-
	II	1 1	Magenta Magenta	83.6 52.0	–	–	–	1	Green	52.0
19	I	1 1	Yellow Magenta	90.7 87.0	1	Yellow	90.7	1	Green	90.7
	II	1	Magenta	52.0	-	-	-	1	Green	52.0
20	I	1 1	Yellow Magenta	90.7 87.0	1	Yellow	90.7	1	Green	90.7
	II	1	Magenta	54.0	-	-	-	1	Green	54.0
21	I	1 1	Yellow Magenta	90.2 90.0	1	Yellow	90.2	1	Green	90.2
	II	1	Magenta	52.0	-	-	-	1	Green	52.0
22	I	1 1	Magenta Blue	85.1 90.7	-	-	-	1	Green	90.7
	II	1	Blue	98.2	-	-	-	1	Green	98.2
23	I	1	Yellow	87.0	-	-	-	1	Green	87.0
	II	1	Magenta	52.0	-	-	-	1	Green	52.0
24	I	-	-	-	-	-	-	-	-	-
	II	1	Blue	98.2	-	-	-	1	Green	98.2
25	I	1	Yellow	90.6	1	Yellow	90.6	1	Green	90.6
	II	1	Yellow	98.0	-	-	-	1	Green	98.0
26	I	1	Blue	88.9	-	-	-	-	-	-
	II	1	Magenta	98.0	-	-	-	1	Green	98.0
27	I	1	Yellow	87.0	-	-	-	1 1	Green Green	87.0 72.7
	II	1	Blue	90.0	-	-	-	1	Green	90.0
28	I	1	Yellow	81.5	-	-	-	1	Green	81.5
	II	1 1	Yellow Magenta	96.0 52.0	- -	- -	- -	1	Green	96.0

2. The developed visible and fluorescent spots/bands were recorded and their hR_f values were calculated as following:

hR_f = (Distance travelled by solute / Distance travelled by solvent) x100

The results obtained were tabulated in Table 3 and 4.

Results and Discussion

Analysis by thin layer chromatography

Mixture of four colors, that is, cyan, magenta, yellow and black (CMYK) of twenty eight toner samples have been analyzed (Mizarchi *et al.*1998) using thin layer chromatography (TLC) and results obtained were observed in visible light and ultra violet light (short and long wavelength). The results have been interpreted on the basis of differences in the number, color and hR_f values of spots.

In solvent I, twenty one toner samples were differentiated from each other on the basis of the differences in the number, color and hR_f value of the developed spots in the day light and ultra violet light (short and long wavelength). Remaining seven samples were put into three groups on the basis of similar number, color and hR_f value of the spots.

In solvent II, the sixteen samples were differentiated from each other on the basis of the differences in the number, color and hR_f value of the developed spots in the day light and ultra violet light (short and long wavelength) . Remaining twelve samples were differentiated into six groups as they had similar number, color and hR_f value of the spots and were put into.

Analysis by high performance thin layer chromatography

Two solvent systems [I and II] were used for the analysis of colored toners. In both solvent systems I and II all toner samples were differentiated from each other on the basis of the differences in the number, color and hR_f value of the developed spots in the visible light at 540 nm (Table 5 and 6).

TLC has successfully differentiated toner samples on the basis of the differences in the number, color and hR_f value of spots. However, some toner samples were not classified because they had similar chromatographic profiles. This might because of two reasons; first being the same type of dye might have been used by various manufacturers during ink formulation. Secondly, the toner ink might have been manufactured in bulk by one company and distributed under different brand names. For better differentiation and classification, the samples were analyzed by HPTLC and

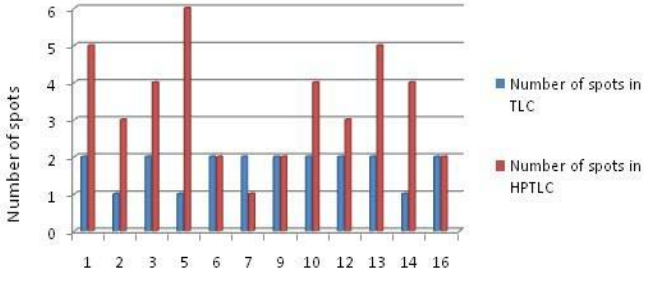

Figure 3: Differentiation of different models of same make by TLC and HPTLC.

Table 5: High Performance Thin Layer Chromatography of Toners at 540 Nm with Solvent System I.

Sample no	No of spots	hR_f values
1	5	64,68,78,84,100
2	3	73,81,84
3	4	63,80,81,84
4	6	16,37,42,61,70,82
5	6	17,22,33,39,77,82
6	2	63,75
7	1	63
8	4	47,53,68,80
9	2	74,81
10	4	66,72,83,86
11	3	74,84,87
12	3	74,80,85
13	5	73,80
14	4	65,73,82,85
15	6	24,54,61,75,84,100
16	2	68,81
17	4	32,73,80,83
18	3	14,77,85
19	5	23,73,78,80,99
20	5	73,81,86,91,99
21	4	77,85,90,99
22	3	73,81,99
23	4	61,73,80,99
24	3	66,79,99
25	4	77,86,89,98
26	4	80,86,89,99
27	3	77,86,99
28	8	10,30,48,61,74,85,89,99

encouraging results were obtained. More spots were detected when the chromatograms were scanned at 540 nm in absorption/ reflectance mode in UV-VIS spectrophotometer. This was due to the high sensitivity of the densitometer which has detected even those spots which were not visible to the naked eyes. It was interesting to found that with increased number of spots different models of the same make were also differentiated (Figure 3). In future studies bulk samples of different ink types could also be analyzed by HPTLC.

Table 6: Differentiation of toner samples by TLC and HPTLC with solvent system I and II.

Chromatographic Method	Solvent I	Solvent II
TLC	21	16
HPTLC (540 nm)	28	28

Conclusion

28 raw and processed toners were analyzed by thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC). Chloroform was used as an extraction solvent. Two solvent systems (code I and II) were used in combination to differentiate toner samples completely. The differentiation was done on the basis of the differences in the number, color and hR_f value of the developed spots. However, significant results have been obtained from the HPTLC chromatograms. It is possible to distinguish toner cartridges on the basis of the differences in the number, color and hR_f value of the spots.

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