High-Performance Thin Layer Chromatography In The Pharma Industry

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ABSTRACT:

Chromatography is routinely employed in pharmaceutical industries for the quantification of drugs and other phrmaceuticals. Among different kinds of chromatographic techniques, high performance thin layer chromatography (HPTLC) had gained popularity because of its speed, versatility, applicability and ability to analyse more than 100 samples parallely. In view of its potential applications, researchers around the world had exploited this technique for the analysis of pharmaceuticals. Here we summarized research work carriedout on 63 formulations, among which 38 formulations contain one active ingredient where as 25 formulations possess two active substances. This review will helpto get a comprehensive understanding of the utility of this technique in pharmaceutical analysis and also for scientists interested to work on HPTLC for their analytical purposes.

KEYWORDS: HPTLC, Pharmaceutical analysis, Method development, Active ingredient,pharmaceuticalformulation.

I. INTRODUCTION

Planar chromatography, or flat-bed chromatography, would be another name for all of it.HPTLC is risk-free and multiple detections can be made without repeating chromatograms. It is quite a lot times faster and economical than HPLC, another liquid chromatography technique. HPTLC is used for the analysis of non-volatile organics such as pharmaceuticals, botanicals, forensics, foods, chemicals, active pharmaceutical ingredients, etc. for establishing purity, reverse engineering, impurities, and fingerprint. HPTLC is a stable, quick, rapid, and effective method for quantitative analysis of compounds[1-7].Common criteria for drug estimation take in the quality and therapeutic value of the pharmaceutical product, content, purity, content uniformity, chemical, and and biological availability[8-11].Table1 listsdifferent performance characteristics of the silica gel plate between TLC and HPTLC[12].

Table 1: Performance features of HPTLC and Thin layer chromatography

PARAMETER (Features of HPTLC versus classical TLC)	Classical TLC	HPTLC
Mean particle size	10-12 µm	5-6 µm
Particle size distribution	5 - 20 µm	4 - 8 µm

Pore diameter	40,60,80,100A^0	60 A^0
Plate dimensions	5×10,5×20,10×20,20×20 cm	10×10,10×20,20×20 cm
Plate height	30 µm	12 µm
Layer thickness	0.20-0.25mm ((250 µm)	0.20-0.25 mm
No of samples per plate	< 10	< 36 (72)
Spot size recommended	2-5mm	1mm
Sample volume	1-5µl	0.1 -0.5 µl
Band size recommended	10-15mm	5-10 mm
Band loading	5-10 µl	1-4 µl
Typical separation time	20 - 200 minutes	3 – 20 minutes
Chromatographic plate used	Handmade /pre-coated	Pre-coated
Pre-washing of the plate	Not followed	Must
Typical migration distance	10 – 15 cm	3- 6 cm
Optimum development distance	10-15cm	5-7cm
Detection limits: absorption	1 – 5 ng	100 – 500 pg
Detection limits: fluorescence	50 – 100 pg	5 -10 pg

HPTLC Benefits:

The most basic separation technique available to the analyst today is HPTLC. It is usually thought of like a time machine that speeds up our work and helps us to do several tasks at once, which is impossible with other analytical techniques. The following are some of the benefits of HPTLC.

- Quicker analysis, only 3 to 20 min for finest separation.
- 5 -10 times superior detection sensitivity than TLC.
- Extremely reproducible, sharp bands for quantitative analysis.
- Easy coupling with bioassays, thus predominantlyvaluable for effect-directed analysis.
- In HPTLC, processing the sample and norm at the same time under identical conditions improves analytical precision and accuracy.

- Defined zones can be absorbed by MS (mass spectrometry after evaluation, hence no need to record every run including matrix and background.
- Internal standards aren't needed very often.
- Shorter study times and lower per-analysis costs.
- HPTLC is a simple procedure to learn, and the instrument is simple to use.
- HPTLC used solvents need not be treated previously.
- Annular grade solvents are appropriate in HPTLC.
- HPTLC permits the use of mobile phases with corrosive and UV absorption.
- The equilibrium time of the device is small in HPTLC.
- HPTLC normally doesn't allow for sampling poisoning during a stationary process.

Steps involved in HPTLC:

1. Selection of chromatographic layer 2. Sample and standard preparation
3. Layer pre-washing 4. Layer pre-conditioning 5. Application of sample and Standard 6. Chromatographic development 7. Detection of spots 8. Scanning 9. Documentation of chromatic plate.

HPTLC Plates:

HPTLC automated multiple development (AMD) Plates:

- It contains extra thin layers of 100 µm.
- Developed for automated multiple development (AMD).
- These plates are suited for particularly qualitative and quantitative detection of pesticides.

HPTLC Plates with Spherical Particles:

- Optimized for high-throughput analysis of complex samples.
- Based on spherical silica gel 60 with a particle size of 7 µm and narrow particle size distribution.
- Outstanding selectivity alike to standard HPTLC plates, yet even higher performance and speed with improved detection limits also.

HPTLC Premium Purity Plates:

- These plates are specially designed for demanding pharmacopoeia applications.
- Wrapped in aluminum foil to stopthe deposition of plasticizers from standard wrapping material, which could result in unknown extra zones when utilizing solvent systems of medium polarity.

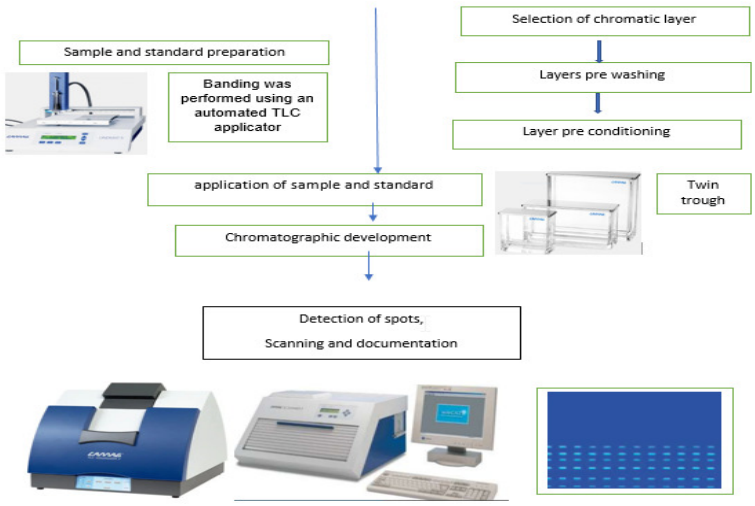

Fig. 1. Explains the instrumentation and steps involved in the HPTLC[13]

II. HPTLC IN THE PHARMA INDUSTRY AND OTHER FIELDS

2.1 In herbal applications of HPTLC:

The HPTLC technique is fast, simple, reliable, and flexible. It's also a great tool for detecting adulterations, botanicals[14-15] lichen substances[6] bamboo leaf flavonoids[17], garlic acid in stem bark[18],Estimation of ascorbic acid in varieties of amla[19],Determination of Glycyrrhizin in Herbal Extract and in Herbal Gel[20], estimation of piperine and vasicine in Vasavaleha[21].Determination of Betulinicacid in Helicteresisora root Extract[23]andevaluating and tracking planting, harvesting, and extraction methods, as well as stability checking. LC Fingerprint is a technique recently introduced by the US and European Pharmacopoeias for the detection of "botanical materials," both of which are extremely complex in nature[24] A fingerprint is a conventional image, such as a photograph, that represents the phytochemical composition of a plant extract or formulation. Fingerprinting may also be used to track batch-to-batch accuracy and stability studies of herbal medicines, dietary supplements, and other products.

2.2 In food analysis:

Foods are usually botanical items, so they are naturally variable and complex. HPTLC can both confirm the identities of complex mixtures and detect adulteration. HPTLC is quick, risk-free, effective, and inexpensive, and it can analyze a lot of samples per day without generating a lot of waste. It is also used for the estimation of isopropylthioxanthone in milk and yogurt[25],mushrooms[26], egg yolk lipids[27].

2.3 In forensic analysis:

Forensic research is a multidisciplinary approach to analyzing crime-related materials and biological samples that employs scientific expertise and advanced instruments. The detection of unknown poisonous substances in lethal intoxication cases is a common yet difficult feature of forensic toxicology. For toxic compounds, HPTLC provides identification, as well

as qualitative and quantitative analysis. For example estimation and identification of heroin in forensic samples[28], new perspectives in the use of ink evidence in forensic science[29].

2.4 In pharmaceutical products:

Many pharmaceutical formulations use HPTLC to ensure safety and efficacy. Pharmaceutical research, biochemistry, and pharmacokinetic studies are only a few of the areas where HPTLC is quickly gaining grip. Pharmaceutical quality control, content uniformity, uniformity test, identity and purity of active ingredients, preservatives in marketed formulations that may be synthetic or herbal, analysis of medicinal plants and herbs, Analysis of pesticide mixtures. HPTLC is used in the estimation ofofloxacin and ornidazole in solid dosage form [30],simultaneous quantitation of paracetamol, diclofenac potassium, and famotidine in tablet formulation[31],determination of etoricoxib and thiocolchicoside in combined tablet dosage form[32] estimation of omeprazole in capsule dosage form[33]estimation of olanzapine in formulations[34]simultaneous analysis of lamivudine, stavudine, and nevirapine in fixed-dose combination tablets[35],quantification of mexiletine hydrochloride in a pharmaceutical formulation[36], determination of salbutamol serum levels in clinical trials[37]and also estimation of many active constituents and also stability testing of different oral dosage forms.

2.5 Cosmetics analysis:

A sunscreen cosmetic could be defined as “any cosmetic product containing UV filters in its formulation to protect the skin from t the solar deleterious UV light, avoiding or minimizing the damage that this radiation might cause on human health” Sunscreen products are usually used across the world due to their benefits of preventing skin from tanning and sunburns.To shield consumer health from any damaging effects, the HPTLC technique can be utilized for the detection of such filters, the identity of which can be further confirmed by the mass spectrometer.

2.6 Specialty chemical analysis with HPTLC:

It is a useful method for determining the purity and impurity of non-volatile organic industrial materials such as dyes, surfactants, pesticides, perfumery compounds, intermediates, and so on. It is much simpler, less expensive, and easier to grasp than other related methods of analysis. HPTLC can be used very well in reverse engineering because it allows for quick comparison. Chemical reactions can be examined in a matter of hours. Complex mixtures, such as biological samples, reaction mixtures, and fermentation broth, the metabolic behavior of microorganisms in the existence of mixed carbon sources[38]can be easily analyzed.

2.7Analysis of HPTLC dyes and intermediates:

Since dyes and intermediates are non-volatile organic substances, they are ideal for HPTLC research. The cost of HPTLC analysis is very low, and the results are "visible." Colored substances arewell-suited to HPTLC analysis. Apart from determining purity and impurity, HPTLC allows for the contrast of various samples or with standards, as well as the study of

competition samples. HPTLC can analyze all types of optical brighteners, intermediates, dyes, and prohibited amines.

2.8 In biotechnology:

HPTLC is a highly flexible analytical technique that provides excellent separation power through the use of precise sample application, software-controlled chromatographic measures, chromatogram creation scanning, and photo documentation.A distinguishing feature of HPTLC is the ability to visually inspect separated samples on the plate. The biotechnology industry is regarded as one of the most research-intensive industries on the planet. As a result, shorter analysis times, lower sample analysis costs per sample, reduced contamination possibilities, and consistently accurate results are needed, which HPTLC provides. HPTLC can analyze several samples at the same time with no chance of cross-contamination. HPTLC also has the advantage of allowing you to evaluate a plate using any particular method and various detection modes (UV, fluorescence, etc.) By combining HPTLC with an MS or other appropriate methods such as NMR, FTIR, ESI, and MALDI, one may classify and/or confirm the chemical structures of analytes under investigation.

2.9 In Purity control:

Testing of vitamin supplements, fruits, vegetables, food items for the existence of pesticides, chemicals by HPTLC is accepted globally due to its ability to characterize tiny molecules.

2.10 Alternative applications of HPTLC:

In recent years, HPTLC has become an internationally accepted functional approach for characterizing small molecules in quality evaluation across the developing world. HPTLC is used to regulate the steroids, purity of chemicals, pesticides, and water. It is also commonly used to analyze water-soluble food dyes, vitamins, and pesticides vegetables, analysis of stem cell lipids[39],determination of identity and quality of botanicals[40] and other foods. HPTLC is effective in identifying forensic chemicals such as abused substances like cannabis[41], tobacco[42],caffeine[43], toxins, food adulterants[44], toxic weapons, and counterfeit drugs. It is also useful in phytochemical analysis of nutraceutical properties of various plant parts used in traditional systems of medicine andestimation of antioxidant activity of nutraceuticals[45]. Fingerprint analysis by HPTLC of naturally occurred products is progressively achieving popularity as the perfect method of screening for adulterants, right from the time of cultivation of raw materials until processing into the required therapeutic dosage form.HPTLC has been used to determine pictogram concentrations and also investigate cases of the drug, alcohol abuse, and chemical warfare in forensics.Common adulterants in so many food supplements and soft drinks like sildenafil and its analogs, detection cannabis in urine samples, preservatives in various pharmaceutical marketed formulations[46]can also be estimated.In separate Tables 2 and 3, brief details containing essential points demonstrating invaluable use in the functional study of HPTLC relating to the compilation of 63 formulations in total, 38 of which contained one active substance and 25 of which contained two active substances is shown. For each formulation, the names of the components, the solvent scheme, the specifics of the plates to be used, and the details of

quantitation demonstrating densitometric evaluation are shown.Table2 shows a compilation of 38 formulations each containing one active substance, as shown below.

Table2: Formulation containing one substance

S.No.	Product	Solvent-System	Plate	Quantitation
1	Alprazolam	Chloroform: ethanol (9:1)	Silicagel60F_{254}	Densitometric evaluation at 225nm
2	Amlodipine besylate	Methanol:ethylacetate:25%ammonia (5:6:0.5)	Silicagel60F_{254}	Densitometric evaluation at 366nm
3	Aminacrine HCl	Ethyl acetate: ammonia (17:2)	Silica	Fluorescence densitometry
4	Bambuterol HCl	Toluene:ethylacetate:methanol: glacial acetic acid (5:2:3:0.2)	Silicagel60F_{254}	Densitometric evaluation at 200nm
5	Bromo hexane HCl	Toluene:chloroform:methanol (7:2:0.5)	Silicagel60F_{254}	Densitometric evaluation at 211nm
6	Cetirizine HCl	Chloroform: methanol (4:1)	Silicagel60F_{254}	Densitometric evaluation at 232nm
7	Caffeine in food materials	Chloroform: acetone (88:12)	SilicagelGF_{254}	Scanner at 275nm
8	Dexamethasone	Chloroform:ethylacetate:methanol (3:7:0.5)	Silicagel60F_{254}	Densitometric evaluation at 232nm
9	Diazepam	Chloroform:ethylacetate (3:1)	Silicagel60F_{254}	Densitometric evaluation at 232nm
10	Enalapril maleate	Ethylacetate:ethanol:acetone (6:5:2)	Silicagel60F_{254}	Densitometric evaluation at 212nm
11	Escitalopram oxalate	Toluene:ethylacetate:triethylamine (14:7:6)	Silicagel60F_{254}	Densitometric evaluation at 240nm
12	Ezetimibe	Chloroform:glacialacteticacid: n-butyl acetate	Silicagel60F_{254}	Densitometric evaluation at 251nm
13	Estriol in serum of a pregnant woman	Toluene:dioxane:methanol (8:2:1)	Silicagel60F_{254}	Fluorescence measurement at 313/400nm
14	Folic acid	Ethanol:25%ammonia:1-propanol (6:2:2)	Silicagel60F_{254}	Densitometric evaluation at 254nm
15	Fluphenazine in plasma	Toluene: acetone (6:3)	Silica gel	Fluorimetry
16	Glibencamide	Chloroform: ethanol (15:1)	Silicagel60F_{254}	Densitometric evaluation at 234nm
17	Glimepiride	Chloroform:cyclohexane:glacial aceticacid:ethanol (9:9:1:1)	Silicagel60F_{254}	Densitometric evaluation at 254nm
18	Hydrochlorothiazide	Toluene:methanol:25%ammonia (6:4:0.1)	Silicagel60F_{254}	Densitometric evaluation at 232nm
19	Levonorgestrol	Chloroform:hexane:methanol (12:1:4)	Silicagel60F_{254}	Densitometric evaluation at 232nm
20	Lisinopril	Chloroform:hexane:methanol (12:4:1)	Silicagel60F_{254}	Densitometric evaluation at 200nm
21	Loperamide HCl	Chloroform:methanol:formic acid (8.5:1:0.5)	Silicagel60F_{254}	Densitometric evaluation at 200nm
22	Loratadine	Diethylether:diethylamine (4:0.1)	Silicagel60F_{254}	Densitometric evaluation at 252nm
23	Nebivolol HCl	Toluene:ethylacetate:methanol: formic acid (8:6:4:1)	Silicagel60F_{254}	Densitometric evaluation at 285nm
24	Norethisterone	Chloroform:cyclohexane:glacialacetica cid:ethanol (9:9:1:1)	Silicagel60F_{254}	Densitometric evaluation at 249nm
25	Olanzapine	Methanol:ethylacetate (4:1)	Silicagel60F_{2}	Densitometric

			$_{54}$	evaluation at 283nm
26	Perindopril erbumine	Toluene:methanol:glacialaceticacid (4:6:0.1)	Silicagel60F_{254}	Densitometric evaluation at 200nm
27	Pioglitazone	Toluene:methanol:25%ammonia (7:3:0.1)	Silicagel60F_{254}	Densitometric evaluation at 270nm
28	Piroxicam	Chloroform:hexane:methanol (12:4:1)	Silicagel60F_{254}	Densitometric evaluation at 363nm
29	Prednisolone	Chloroform:ethylacetate:methanol (3:7:0.5)	Silicagel60F_{254}	Densitometric evaluation at 249nm
30	Prochlorperazine maleate	Methanol: n-hexane (7:5)	Silicagel60F_{254}	Densitometric evaluation at 285nm
31	Paracetamol in serum	Hexane:acetone:ethanol (6:3:1)	Silica	Densitometry
32	Rouvastatin	Chloroform:methanol:toluene (3:1:1)	Silicagel60F_{254}	Densitometric evaluation at 311nm
33	Salbutamol sulphate	Chloroform:methanol (1:3)	Silicagel60F_{254}	Densitometric evaluation at 232nm
34	Salbutamol in human plasma	Ethylacetate:chloroform:methanol (60:40:1)	Silica	Densitometry at 650nm
35	Sulfa drugs (around 20 analogues)	Ethyl acetate: ammonia (99:1)	Silica	Densitometry in fluorescence mode at 366nm
36	Telmisartan	Methanol: n-hexane(2:5)	Silicagel60F_{254}	Densitometric evaluation at 285nm
37	Terbutaline sulphate	2-propanol:cyclohexane:formicacid (6.5:2.5:0.5)	Silicagel60F_{254}	Densitometric evaluation at 200nm
38	Vitamin B_1 in formulations	Methanol:25%ammonia:aceticacid:chloroform(18:2:1:1)	SilicagelGF$_{254}$	TLC scanner 366/400 nm

Compilation of 25 formulations containning two active substances is shown in Table 3 as indicted bellow.

Table 3: Formulation containing two active substances

S.No.	Product	Solvent-system	Plate	Quantitation
1	Amlodipine besylate and benazepril HCl	Ethylacetate:methanol:25% ammonia (8:2:1.5)	Silica gel 60F_{254}	Densitometric evaluation at 244nm
2	Atenolol and nifedipine	Toluene:2-propanolol (7.5:2.5:0.1)	Silica gel 60F_{254}	Densitometric evaluation at 235nm
3	Amlodipine besylate and enalapril maleate	Chloroform:methanol:glacial acetic acid (8.5:1.5:1)	Silica gel 60F_{254}	Densitometric evaluation at 230nm
4	Amlodipine besylate and lisinopril	n-butanol:methanol:25% ammonia (3:1:1)	Silica gel 60F_{254}	Densitometric evaluation at 200nm
5	Amlodipine besylate and ramipril	Ethylacetate:methanol:25% Ammonia(8.5:1:0.5)	Silica gel 60F_{254}	Densitometric evaluation at 280nm
6	Amlodipine besylate and nebivolol HCl	Glacial acetic acid:water:methyl isobutyl ketone (25:25:50)	Silica gel 60F_{254}	Densitometric evaluation at 210nm
7	Amlodipine besylate and atorvastatin calcium	Chloroform:methanol:acetone:25% ammonia (18:6:10:3)	Silica gel 60F_{254}	Densitometric evaluation at 240nm
8	Atorvastatin calcium and	Toluene:methanol:acetone: triethylamine (7:2:1:0.2)	Silica gel 60F_{254}	Densitometric evaluation at 257nm

	ezetimibe			
9	Atorvastatin calcium and ramipril	Toluene:ethylacetate:methanol: glacial acetic acid (5:4.5:0.5:0.5:0.1)	Silica gel $60F_{254}$	Densitometric evaluation at 210nm
10	Chlorpromazine and thioridazine	Double development toluene: acetone (5:5) toluene:acetone:ammonia (50:50:2.4)	Silica gel $60F_{254}$	Densitometric evaluation at 365nm for chlorpromazine and 375nm for thiopridazine
11	Chlordiazepoxide andclidinium bromide	Acetone:methanol:water:0.2M HCl (14:4:1:1)	Silica gel $60F_{254}$	Densitometric evaluation at 200nm
12	Hydrochlorothiazide and lisinopril	n-butanol:methanol:water (3:1:1)	Silica gel $60F_{254}$	Densitometric evaluation at 200nm
13	Hydrochlorothiazide and nebivolol HCl	Ethyl acetate :methanol: glacial acetic acid (6:5:1:0.5)	Silica gel $60F_{254}$	Densitometric evaluation at 285nm
14	Hydrochlorothiazide and ramipril	Toluene:acetone:methanol:25% ammonia (6:4:3:1)	Silica gel $60F_{254}$	Densitometric evaluation at 210nm
15	Hydrochlorothiazide and propranolol HCl	Toluene:methanol:ammonia	Silica gel $60F_{254}$	Densitometric evaluation at 280nm
16	Isoniazidand thioacetazone	Chloroform:methanol:glacial acetic acid (9:1:0.1)	Silica gel $60F_{254}$	Densitometric evaluation at 272nm
17	Ketoprofen and propyphenazone	Toluene: acetone (7:3)	Silica gel $60F_{254}$	Densitometric evaluation at 265nm
18	Metoclopramide and paracetamol	Ethyl acetate:acetone:ammonia (6:4:0.1)	Silica gel $60F_{254}$	Densitometric evaluation at 300nm
19	Metronidazole and furazolidone	Chloroform:acetone:methanol (10:1.6:0.4)	Silica gel $60F_{254}$	Densitometric evaluation at 280nm
20	Montelukast sodium and levocetirizine HCl	Ethyl acetate:methanol:25% ammonia (7:1.4:0.7)	Silica gel $60F_{254}$	Densitometric evaluation at 231nm
21	Paracetamol and methocarbamol	Chloroform:methanol:glacial acetic acid (9.5:0.5:0.2)	Silica gel $60F_{254}$	Densitometric evaluation at 275nm
22	Sulphamethoxazole and trimethoprim	Chloroform: ethanol (9:1)	Silica gel $60F_{254}$	Densitometric evaluation at 284nm
23	Simvastatin and excetimibe	n-hexane: acetone (12:8)	Silica gel $60F_{254}$	Densitometric evaluation at 251nm
24	Tinidazole and diloxanide furoate	Toluene:acetone:ammonia (8:2:0.5)	Silica gel $60F_{254}$	Densitometric evaluation at 280nm
25	Terbutaline sulphate and bromhexine HCl	2-propanol:cyclohexane:formic acid (6.5:2.5:0.1)	Silica gel $60F_{254}$	Densitometric evaluation at 200nm

III. CONCLUSION

The pharma industry has observed a rise in the use of HPTLC alone or in combination with other techniques such as FTIR, MS[47]for the determination of formulations and bulk drugs and flavonoids content in some leaf extracts of syzygiumcumini[48] and phytocmecalscreeing and HPTLC fingerprinting of extracts of Thujaoxidentalis[49]and some classical Ayurvedic Preparations[50]. HPTLC is also being utilizedmagnificently in fields of biomedicine, biochemistry with a growing trend in its application in modern agriculture for estimating pesticide residues in fruits and vegetables. HPTLC is also often seen in pharma fieldand clinical research, analysis of medicinal plants and conventional medicines, analysis of food, feed, commodities, and dietary supplement, environmental, cosmetic, toxicological, and forensic aspects, herbal and plant analysis, and detection of free radical scavenging

activity[51,52].This HPTLC method was developed and validated as per the guidelines of ICH[53-60].The critical challenges concerned with column-based hyphenations are capital costs, approaches for coping with massive amounts of information produced by the application, which increases the complexity of instrumentation, and operational difficulties.

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