DEVELOPMENT OF A THIN LAYER CHROMATOGRAPHIC METHOD FOR SIMULTANEOUSLY SCREENING MULTIPLE DRUGS IN SERUM

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ABSTRACT:

Background: Multiple drug screening is required to find out if a drug or a cocktail of drugs is the cause of illness or death. A thin layer chromatography was developed for this purpose as an alternative method for laboratories lacking in high technological chromatography.

Methods: Drug spiked serums were extracted, spotted on a commercial TLC plate, developed in a 250 mL beaker, and colorized by reacting with certain reagents.

Results: The turnaround time of this method was about 1 hour. Twenty drugs could be identified after reacting with 2 or more detection reagents. Adding iodine in the reagent, instead of using iodine vapor, could enhance the detection capacity. In contrast, the plate drying temperature had no impact on the reaction with ninhydrin.

Conclusions: The developed TLC method can be used to screen several drugs simultaneously. It is simple and rapid for preliminarily identifying blood drugs in intoxicated or over-dosed cases. The results can narrow down the suspected drug(s) which can be confirmed by even a single drug immunoassay method available in most hospital laboratories.

Keywords: Thin layer chromatography; Drug screening; Detection reagent

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INTRODUCTION

A drug or a cocktail of drugs is usually a suspected cause of emergency, accidental, criminal, and abusing cases [1, 2], thus, the drug screening is often ordered in such cases. While necessary, most laboratories are unable to perform the drug screening due to lack of expensive and sophisticated equipment and expertise [3]. Without a drug screening result, it may plausibly compromise the acquisition of appropriate data and the correct interpretation. Consequently, physicians may be reluctant to take risks whenever a minimum of uncertainty exists, leading to over-treatment and inappropriate disposal [4-6]. For psychotropic drugs, no drug screening result will lead to the lack of clarity which will continue to impair the design and targeting of appropriate and effective preventive interventions to end the reliance on prevailing manner of death distinctions for classifying fatal drug intoxications [5, 7, 8]. Therefore, the drug screening results have not only clinical benefits but also pivotal judicial, social, personal, and economical consequences [9].

To make a drug screening serviceable without an expensive instrument in most hospital laboratories, a simple thin layer chromatographic method was developed.

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MATERIALS AND METHODS

TLC plates, chemicals and reagents

The TLC plates; 20 x 20 cm $60F_{254}$, were purchased from Merck KGaA (Darmstadt, Germany). All reagents and chemicals were of analytical grade or better. Potassium iodide and iodine were purchased from Thermo Fisher

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Scientific Inc. (Waltham, *Massachusetts*). Bismuth subnitrate and ninhydrin were purchased from Fluka (Buchs, Switzerland). Other reagents and chemicals were purchased from Merck KGaA (Darmstadt, Germany). All standard drugs were purchased from Sigma-Aldrich Co. LLC. (St. Louis, Missouri). The detection reagents were prepared as previously reported [10-14].

Sample preparation and analysis

One hundred left over serums from routine laboratory tests were anonymously collected. They were screened for drugs by the GC-MS (Agilent technologies, Palo Alto, California). All drug free serums were pooled in a clean brown bottle and were used for preparing drug spiked serums. One mL of serum was extracted by mixing with 500 µL of dichloromethane, 200 µL of saturated NaCl, and 200 µL of 0.1 M sodium carbonate. Five µL of dichloromethane layer was spotted on a 6 x 8 cm TLC plate. The chromatogram was run in the mixture of methanol, dichloromethane, ethyl acetate, and concentrated ammonium hydroxide at the amounts of 2, 8.5, 8.5, and 1 mL, respectively, in a 250 mL beaker for 10 min. Then, the plate was removed from the tank, placed until dried, and followed by dipping thoroughly with a specific detection reagent. After lifted up, the occurring color bands were investigated within 10 min.

RESULTS

The 6 x 8 cm TLC plate could be placed vertically and steadily in the 250 mL beaker. Up to 5 samples could be run simultaneously on a single plate. The mobile phase made clear separation bands within 10 min. No a false positive result was observed. The single drug spiked serums gave results with certain detection reagents as shown in Table 1. Unlike other reagents which gave a unique color with all positive drugs, Marquis gave remarkably colors depending on drugs which is helpful for drug identification. The colors faded down and disappeared within an hour, thus they should be observed within 10 min. The drug screening results from certain mixture of drugs spiked in pooled serum are revealed in Table 2. Drugs detected by GC-MS gave the results detected by TLC method as shown in Table 3.

Table 1 The results of drugs reacted with specific detection reagents

Drug	Detection limit (µg/mL)						Rf
	Ninhydrin	Dragendorff	Iodinated Dragendroff	Methanolic idodine	Marquis	5% Ferric chloride	
Amitriptyrine	2.0	0.5	0.5	0.5	0.5	-	0.70
Amphetamine	0.5	-	2.0	0.5	0.5	-	0.48
Caffeine	-	-	5.0	-	-	-	0.54
Clonazepam	-	-	5.0	-	2.0	-	0.60
Cocaine	-	0.5	0.5	0.5	-	-	0.87
Codeine	2.0	2.0	0.5	0.5	0.5	-	0.50
Dextromethrophan	-	0.5	0.5	0.5	1.0	-	0.43
Diazepam	-	2.0	0.5	0.5	-	-	0.65
Ephedrine	0.5	-	1.0	1.0	5.0	-	0.23
Fenfluramine	1.0	0.5	0.5	2.0	-	-	0.63
Heroin	1.0	2.0	0.5	0.5	1.0	-	0.60
Ketamine	-	5.0	0.5	0.5	-	-	0.85
MDMA	0.5	5	1.0	0.5	0.5	-	0.42
Methadone	2.0	0.5	0.5	0.5	5.0	-	0.75
Methamphetamine	0.5	5.0	1.0	0.5	0.5	-	0.43
Midazolam	-	2.0	0.5	0.5	-	-	0.69
Morphine	1.0	5.0	0.5	0.5	0.5	2.0	0.23
Paracetamol	-	-	-	2.0	-	5.0	0.47
Phentermine	2.0	-	0.5	1.0	1.0	-	0.45
Pseudoephdrine	0.5	-	1.0	2.0	5.0	-	0.25

Table 2 The drug screening results of multiple drug spiked serums

Spiked serum No.	Drugs added in the serum	Concentration of each drug in the serum	Results from Iodinated Dragendroff	Results from Dragendroff reagent	Results from Marquis reagent
1	Cocaine, Caffeine, Methamphetamine	5 µg/mL	Cocaine, Caffeine, Methamphetamine	Cocaine, Methamphetamine	Methamphetamine
2	Cocaine, Caffeine, Methamphetamine	1 µg/mL	Cocaine, Methamphetamine	Cocaine	Methamphetamine
3	Amitriptyline, Diazepam, Heroin	5 µg/mL	Amitriptyline, Diazepam, Heroin	Amitriptyline, Diazepam, Heroin	Amitriptyline, Heroin
4	Amitriptyline, Diazepam, Heroin	1 µg/mL	Amitriptyline, Diazepam, Heroin	Amitriptyline	Amitriptyline, Heroin
5	MDMA, Dextromethrophan, Morphine	5 µg/mL	MDMA, Dextromethrophan, Morphine	MDMA, Dextromethrophan, Morphine	MDMA, Dextromethrophan, Morphine
6	MDMA, Dextromethrophan, Morphine	1 µg/mL	MDMA, Dextromethrophan, Morphine	Dextromethrophan	MDMA, Dextromethrophan, Morphine

Table 3 The drug screening results analyzed by GC-MS vs. TLC method

Sample number	TLC drug screening result	Results from GC-MS
1	Amitriptyline	Amitriptyline
2	Caffeine	Caffeine
3	not detected	Caffeine
4	not detected	Caffeine
5	Codeine	Codeine
6	Codeine	Codeine, Pseudoephedrine
7	Dextromethrophan	Dextromethrophan
8	not detected	Clonazepam
9	Diazepam	Diazepam
10	Midazolam	Caffeine, Midazolam
11	Morphine	Morphine
12	Paracetamol	Paracetamol
13	not detected	Paracetamol
14	not detected	Pseudoephedrine

DISCUSSION AND CONCLUSION

TLC has been established as a standard method for the preliminary identification of drugs in their pure forms by the US national institute of justice [11] and for urine drugs testing by the US national institute on drug abuse [12]. One of its advantages is the ability to detect multiple drugs simultaneously as other sophisticated chromatographic technique can. However, it can be performed with limited resources and has been developed for drug quality and urine drug testing [10, 13-21]. This presentation aimed to develop a TLC method for blood drug screening test and applied it as a hospital laboratory service.

TLC has been criticized as a time-consuming technique [7]. To reduce the turn-around time, the commercial TLC plates were utilized to omit the plate preparation time. Since the TLC plate is a disposable stationary phase [13], the sample extraction could be roughly done. Saturated sodium chloride solution was added to salt out serum protein [22]. The total analytical time from sample preparation to report was around 1 hour so the desired turnaround time for most emergency setting was achievable. Adding a small amount of volatile alkali solvent such as concentrated ammonium hydroxide in the mobile phase, as previously reported [14], could improve the compactness and resolution of occurring bands.

Ninhydrin has been reported for detecting various drugs based upon the plate heating

temperature prior dipping [10]. The developed plate needed to be heated before dipped with ninhydrin otherwise the whole plate became dark brown. But increasing plate heating temperature from 100 to 120 or 160ºC did not increase number of drugs detected. Adding iodine in the reagent, instead of exposure to iodine vapor as recommended [13, 21], enhanced the detection capacity. The iodinated Dragendorff reacted with more drugs than Dragendorff. Additionally, both iodinated reagents gave clear dark brown easily to be detected. The detection limits of some drugs are high (Table 1), thus, this method is not sensitive enough for illegal drug abusing, doping, and workplace drug using tests, but suitable for accident and emergency drug overdose or intoxication cases. Because of its low sensitivity, the method can give false negative when drug concentrations are less than their detection limits (Table 2 and 3). As a matter of fact, the drug screening is essentially designed to quickly eliminate negative samples from further more costly confirmatory testing [7].

The TLC has several attractive features such as ability to detect more than one drug simultaneously, parallel sample processing for high sample throughput, and single use of the TLC plate for minimal sample clean-up [23]. Indeed, it was suggested as an initial screen when the ability to screen inexpensively for a large number of drugs was more important than the degree of sensitivity [12].

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