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PLACKETT-BURMAN DESIGN OPTIMIZED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR DETERMINATION OF METHAMPHETAMINE FROM LABORATORY COAT MATERIALS

(Pengekstrakan Mikro Cecair-Cecair Serakan Gandingan Kromatografi Gas-Spektrometri Jisim yang Dioptimumkan Secara Rekaan Plackett-Burman untuk Penentuan Metamfetamin Daripada Bahan Kot Makmal)

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Abstract

Accumulated drug residues on contaminated clothing put the wearer at adverse health risk. Therefore, monitoring on laboratory coat contamination shall be in place to safeguard the analysts who have routine exposure in forensic laboratory environment. Using methamphetamine as surrogate drug, this study was aimed to detect the presence of methamphetamine from laboratory coat materials through a response surface methodology optimized dispersive liquid-liquid microextraction (DLLME) in couple with gas chromatography-mass spectrometry (GC-MS). In this study, a Plackett-Burman design was used to optimize the DLLME conditions, including the volumes of extraction and dispersive solvents, the duration of vortex agitation, as well as the speed and time of centrifugation. Residues collected upon DLLME procedure was priorly derivatized with trifluoroacetic acid anhydride followed by GC-MS analysis. Seven types of fabric materials which were contaminated with methamphetamine were extracted and compared. From this study, a volume of 685 µL dichloromethane as extraction solvent, 1000 µL 2-propanol as dispersive solvent, vortex agitation for 90 seconds, and centrifugation at 500 rpm for 5 minutes were determined as the optimum conditions for DLLME. Trace methamphetamine residues were successfully extracted and detected from the different types of laboratory coat materials with recovery percentage of at least 45%. The method could be proposed to laboratories for their evaluation on possible contamination to establish baseline for necessary in-house monitoring and decontamination procedures.

Keywords: Illicit drugs, methamphetamine, dispersive liquid-liquid microextraction, Plackett-Burman design, contaminated laboratory coat

Abstrak

Sisa-sisa dadah yang terkumpul pada pakaian tercemar membawa risiko mudarat kepada pemakai. Justeru, pemantauan pencemaran kot makmal harus diberi perhatian untuk melindungi penganalisis yang mempunyai pendedahan rutin dalam persekitaran makmal forensik. Dengan menggunakan metamfetamin sebagai dadah surogat, kajian ini bertujuan untuk mengesan kehadiran metamfetamin daripada bahan kot makmal melalui pengekstrakan mikro cecair-cecair serakan (DLLME) yang digandingkan dengan kromatografi gas-spektrometri jisim (GC-MS) yang telah dioptimumkan dengan kaedah gerak balas permukaan merangkumi isipadu pelarut pengekstrakan dan pelarut serakan, tempoh pengadukan pusaran, serta kelajuan dan masa pengemparan. Sisa-sisa yang dikumpulkan setelah prosedur DLLME telah diterbitkan terlebih dahulu dengan asid trifluoroasetik anhidrida diikuti dengan analisis GC-MS. Tujuh jenis bahan fabrik yang telah dicemari dengan metamfetamin telah diekstrak dan dibandingkan. Daripada kajian ini, 685 µL diklorometha sebagai pelarut pengekstrakan, 1000 µL 2-propanol sebagai pelarut serakan, pergerakan vorteks selama 90 saat, serta pengemparan pada 500 rpm selama 5 minit telah ditentukan sebagai keadaan optimum bagi DLLME. Sisa-sisa surih metamfetamin telah berjaya diekstrak dan dikesan daripada jenis bahan kot makmal yang berbeza dengan peratusan pemulihan semula sekurang-kurangnya 45%. Kaedah tersebut boleh dicadangkan kepada makmal-makmal untuk penilaian mereka terhadap pencemaran yang berkemungkinan untuk menetapkan garis dasar untuk pemantauan dalaman dan prosedur penyahcemaran yang diperlukan.

Kata kunci: Dadah haram, metamfetamin, pengekstrakan mikro cecair-cecair serakan, rekaan Plackett-Burman, kot makmal tercemar

Introduction

Handling and processing illicit drugs in a drug testing laboratory are known to have potentially contaminated various surfaces within the laboratory [1-3]. It is important to note that illicit drugs often with unknown formulation or synthesis pathway could pose serious health and safety risk to individual who had direct or even indirect contact with the constituents. Besides the cooks and operators of a clandestine drug laboratory, other vulnerable groups such as occupants residing around the clandestine laboratory, law enforcement personnel who investigate drug scene or even the laboratory analysts who perform routine analyses on illicit drugs or their associate chemical are all exposed to additional risk capable of leading to chronic health effects [4, 5]. Consensus standard for occupational exposure limits for common illicit drugs and their precursors for forensic laboratory settings are yet to be established although a study by the National Institute for Occupational Safety and Health (NIOSH) had reported that the detectable cocaine, fentanyl, heroin, and methamphetamine are present at nanogram level, respectively, in the air and on surfaces within drug testing laboratories [2].

In relation to illicit drug contamination to the public, investigations in waste waters and surface water environment to explore the health effects and the trends of drug usage in the local communities were reported [6-8]. Studies were also conducted on various daily high contact surfaces that were found to contain detectable levels of illicit drugs [9, 10], suggesting the possibility of transferring and persisting of these substances. Airborne particles of illicit drugs were also found to be persisting in the forms of residual powder or condensed aerosol [11-13]. Clandestine drug laboratories, in the stage of active or dormant, were contaminated following the activities during cooking and subsequent processing steps [4, 14], where the surface deposition of drug was reported to be as high as 860.0 µg of methamphetamine on a surface of 100 cm^2 [15]. It was also important to note that the illicit drugs and by-products during the clandestine activities could have contaminated the soil, water, and air within or proximate areas to a clandestine drug laboratory site [4, 16].

In the setting of forensic testing laboratories, there are personnel who face higher risk of drug exposure, especially individuals who handle and perform routine drug analyses [1-3]. Residual smoke pollutant could be present on the surfaces and in dust which tend to be released again into the gas phase interacting with other

contaminants and oxidants to form secondary pollutant [17, 18]. Studies have shown that the aerosolized release of illicit drug substance was evident, and drug particles could spread throughout the laboratory via touch, direct transfer, and/or suspension of particulate in the air [1-3], where the analytical balance and its surrounding area were most contaminated. Furthermore, it has been reported that the drug substance can adhere to clothing and other fabrics, even at low and sustained levels of air concentration [17, 18]. This could lead to re-exposure through contact with these contaminated materials and clothing [18].

In the United States, a study conducted by Sisco and Najarro [3] involving over 700 samples from 20 laboratories showed that the contamination levels were at the highest within the drug unit, measured with at least 10 nanograms levels in nearly all sampled areas. Cocaine and heroin were detected more frequently with the highest surface concentrations in these laboratories [3]. These two drugs were highly prevalent on the surfaces and in the air of drug testing laboratories as indicated by NIOSH [2], where 11 out 12 tested laboratories were found to contain cocaine, and heroin was found in nine laboratories. Additionally, airborne fentanyl and methamphetamine were found in four and three laboratories, respectively. All the targeted drugs i.e. cocaine, heroin, fentanyl, and methamphetamine were detected on the bench surfaces in all the twelve laboratories, except one laboratory without detectable level of methamphetamine [2]. In Australia, residues of illicit drugs were detected in police stations [16, 19], where handling of drug evidence could contribute to an elevated background level at the police stations at a level less than 50 ng. Contamination in crime scene vans and storage lockers, report preparation desks, and toxicology units were also reported [1].

Although studies have investigated on various areas within a drug testing laboratory and toxicology units for the presence of drugs, contamination level of laboratory coat worn as personal protection equipment was understudied. This study was initiated to provide some insight on drug contamination levels from a frequently overlooked perspective, specifically from the laboratory coat worn by the laboratory personnel, using methamphetamine as an indicator of the degree of contamination, since methamphetamine is among the most widely abused drug as indicated by its users followed by opiates, amphetamine-type simulant (ATS), cannabis and others [20].

Applications of DLLME in analytical chemistry included the examination of narcotics, illicit drugs, hallucinogens, and cannabinoids [21-23]. In DLLME, the mixing of extraction and dispersive solvents and addition to the aqueous sample solution led to the formation of tiny droplets dispersed throughout the aqueous phase. The creation of a large contact area allows for a fast equilibrium within short extraction time. Additionally, vortex agitation contributes to a rapid partition equilibrium while the centrifugation step facilitates the phase separation after extraction. Upon DLLME, the enriched analytes can be collected with a microsyringe from the sedimented phase and subjected to further examination [22-24].

Using dispersive liquid–liquid microextraction (DLLME) and gas chromatography-mass spectrometry (GC-MS), we reported a Plackett-Burman Design (PBD) for method optimization which was implemented for the determination of methamphetamine from the laboratory coat materials. PBD is a two-level factorial design that only requires N=4t runs (a multiple of 4 for the number of runs) to separate main effects from the interaction effects. Previous studies have also suggested its applicability and practicality in enhancing an analytical technique, particularly during the optimization step [25, 26]. It is hoped that the study would aid in proposing an analytical method to provide a quick snapshot on possible laboratory coat contamination or as a long-term monitoring program incorporated into the quality management system for appropriate laboratory clothing decontamination procedures.

Materials and Methods

Materials and chemicals

Methamphetamine hydrochloride (99.2% purity) was supplied by the Department of Chemistry Malaysia. Tetradecane standard, as the internal standard, was purchased from Dr. Ehrenstorfer GmbH (Augsburg,

Germany). Chromatography-grade acetonitrile, chloroform, dichloromethane, methanol, and 2-proponal were purchased from Merck (Whitehouse Station, NJ, USA). Trifluoroacetic acid anhydride (TFAA) (>99%) used as derivatization agent was purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals used as they are. A stock solution of methamphetamine was prepared at 50 mg/mL by dissolving 500 mg of methamphetamine standard in methanol and diluting to 10 mL in a volumetric flask. Working standard solutions were prepared by appropriately diluting the stock solution into the pre-defined concentration levels.

Laboratory coat materials

Seven types of fabric materials commonly used for making laboratory coat were obtained from local clothing retailer. These materials were then cut into squares (10 x 10 cm). Prior to the analyses, the laboratory coat materials were washed, dried, and kept in a resealable plastic bag.

Plackett-Burman design of DLLME optimization

The choice of solvents is crucial in DLLME for good extraction efficiency. A preliminary experiment on the selection of extraction (chloroform and dichloromethane) and dispersive (acetonitrile, methanol, and 2-propanol) solvents were firstly carried out. For each combination, 300 µL of each extraction solvent was used in coupled with 300 µL of dispersive solvent which was adapted from the study [21]. The combination which gave the high extraction efficiency was determined through the determination of peak area ratio, considering the peak area of methamphetamine against the peak area of internal standard. Based on the outcome, DLLME was optimized through a PBD using Minitab® 18 software (Minitab Inc., State College, PA, USA). Five factors, covering the volume of extraction solvent, volume of dispersive solvent, duration of vortex agitation, centrifugation time, and centrifugation speed were evaluated with 2 replicates and 2 center points, totaling at 26 runs as demonstrated in Table 1.

Table 1. Plackett-Burman design with five factors of variables.

Factor	Low (-1)	Medium (0)	High (+1)
Volume of extraction solvent (µL)	110	555	1000
Volume of dispersive solvent (µL)	100	550	1000
Duration of vortex agitation (s)	0	45	90
Centrifugation time (min)	5	7.5	10
Centrifugation speed (rpm)	500	1250	2000

Recovery study: Sample preparation procedure

A volume of 100 µL methamphetamine at three different concentrations, namely 5 µg/mL, 15 µg/mL, and 30 µg/mL, was separately spread over the defined area on the laboratory coat materials following the method used in surface contamination study conducted before [27]. The deposited solution was then allowed to dry for 5 mins prior to extraction, leading to the presence of defined masses of methamphetamine (0.5 µg, 1,5 µg and 3.0 µg) on each laboratory coat material. It was noted that the maximum acceptable amounts of surface methamphetamine were varied among the countries worldwide, ranging from 0.5 µg to 4.0 µg per an area of 100 cm^2 [14]. Upon drying, a 7 mL aliquot of 4% (m/v) of sodium hydroxide solution was added to culture tube with the cloth samples followed by 5 mins sonication (200 W) for basic extraction of the drug. The sonicated samples were then transferred into a 10 mL glass syringe, where the syringe plunger was depressed as much as possible to dispense the solution into a 20 cm glass culture tube. The step was repeated by transferring the laboratory coat materials from the syringe into the culture tube followed by an additional of 7 mL 4% of NaOH solution. The sample solution was again dispensed and combined. Prepared solution was then subjected to the optimized DLLME procedure. Subsequently, the extraction phase-dispersed particles found sedimented at the bottom which then were collected and evaporated to dryness under a gentle stream of purified nitrogen gas.

Sample derivatization by TFAA

The sample derivatization procedure was adapted from Abdullah and Miskelly [27]. For derivatization, the residue was added with 100 µL of dichloromethane followed by 100 µL of ethyl acetate and 50 µL of TFAA. The sample was then subjected to a 30-min incubation at 38°C in an oven. Upon incubation, the excessive solvents and TFAA were carefully evaporated using a gentle stream of nitrogen flow. The residue was then reconstituted with 1 mL of ethyl acetate containing 0.25 µL/mL of tetradecane.

GC-MS analysis

Gas chromatographic analyses were performed using a 7890B GC system equipped with a 5977-mass selective detector (MSD) and split/spitless injector (Agilent Technologies, Santa Clara, CA, USA). A HP-5MS capillary column (30 m x 0.32 mm 0.25 µm film thickness) was used for separation and purified helium gas as the carrier gas was flowed at a constant rate of 1.0 mL/min. The front inlet was set at 250°C while the oven temperature was programmed as follows: 70°C for 2 mins, increased at 20°C/min to 280°C, and then hold at 280°C for 2 mins. MassHunter Workstation Software (Version 3.1, Agilent Technologies, Santa Clara, CA, USA) was used for data acquisition and interpretation, where the results were at a rate of 1.0 sec/scan and the mass spectra was collected in scan mode from m/z 41 to m/z 500. The resulting peaks were confirmed through the comparison of retention times and determination by the National Institute of Standards and Technology (NIST) MS Search Version 2.0 along with the NIST mass spectral library (NIST17) (Gaithersburg, MD, USA).

Upon reconstitution, the sample vial was flushed with nitrogen gas, capped, and shaken well prior to GC-MS analysis. Utilizing the optimized DLLME conditions, the recovery percentages (% recovery), calculated by dividing the mass of methamphetamine determined from the standard calibration curve with the known amount of methamphetamine in percentage, and the relative standard deviations (%RSD) from the seven types of laboratory coat materials were evaluated and compared.

Results and Discussion

Selection of extraction and dispersive solvents

In a DLLME technique, extraction solvent and dispersive solvents with adequate water miscibility and higher density than water are crucial to achieve a good extraction efficiency and good GC chromatogram [28]. The extraction solvent, i.e. dichloromethane has high capability for extracting analytes with a low water solubility while being soluble in the dispersive solvent. The phase separation requires a significant density difference between the extracting solvent and water [29]. In this study, extraction efficiencies of each combination of extraction and dispersive solvents were compared and evaluated (Figure 1). It was found that a combination of dichloromethane (extraction solvent) and 2-propanol (dispersive solvent) demonstrated the highest extraction efficiency for methamphetamine at a peak area ratio of 6.127 as compared to other solvent combinations. Dichloromethane demonstrated its good extraction capability for the target analytes, and able to form an emulsion with the presence of the dispersive solvent [30,31]. On the other hand, the dispersive solvent (2-propanol) has low interfacial tension, allowing for the dissolution of both organic phase (extraction solvent) and the aqueous phase (sample solution) [32].

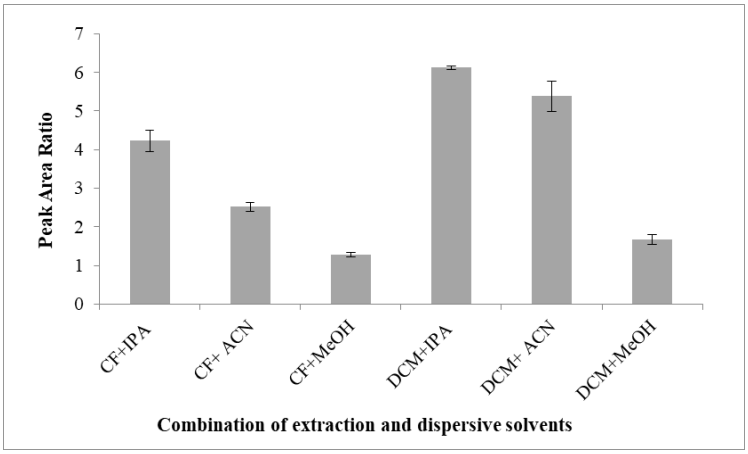

Figure 1. Extraction efficiencies with various combination of extraction and dispersive solvents at 1:1 ratio (n=7) (CF: chloroform; DCM: dichloromethane; IPA: 2-propanol; CAN: acetonitrile; MeOH: methanol)

PBD of DLLME optimization

A response surface methodology, namely the PBD was used to determine the experimental settings involving five variables for the greatest extraction efficiency of methamphetamine. The design allowed for the collection of the maximum amount of information with a minimum number of analyses [33]. The influences of each factor, including volume of the extraction solvent (x_1), volume of dispersive solvent (x_2), duration of vortex agitation (x_3), centrifugation time (x_4), and centrifugation speed (x_5), were tested. The collected experimental data were utilized to develop an estimate for a model that can adequately estimate the response variable given in the second order polynomial function as in the following equation:

Methamphetamine peak area: $$-3.202 + 0.03356x_1 + 0.002848x_2 + 0.00032x_3 + 0.3514x_4 - 0.000946x_5 - 0.00028x_1 * x_1 + 0.000002x_1 * x_2 + 0.000052x_1 * x_3 - 0.000458x_1 * x_4 + 0.000001x_1 * x_5 - 0.000002x_2 * x_3 - 0.000558x_2 * x_4$$

Analysis of variance (ANOVA) statistical test revealed that the fitted response surface model of second order was highly significant with F-value = 182.31 (p<0.001). The model explained 99.41% of the variation, with a predicted R^2 of 97.64%. The large predicted R^2 values suggested a good predictive ability of the model. Figure 2 shows the Pareto chart of the standardized effects comparing the relative magnitude and the statistical significance of main, square, and interaction effects in this study. The reference line at standardized effect of 2.16 indicated the significant effect as a significance level of 0.05. From the Pareto chart, the largest effect was contributed by the squared term for volume of extraction solvent followed by main effects of the volume of extraction volume and the time of vortex agitation.

The association between the detection of methamphetamine and each term was then determined and Table 2 demonstrates the output of the ANOVA statistical test. By considering a variable to be statistically significant at a 5% level of significance, the coefficient for the linear effect of the variables included extraction volume (p <0.001), vortex time (p <0.001) and centrifugation time (p <0.001) which were highly significant. The centrifugation speed was also statistically significant (p = 0.041), where no significant association between the volume of dispersive solvent (p = 0.375) with the recovery of methamphetamine upon DLLME procedure. The squared terms for the volume of extraction solvent were also significant at the α = 0.05 significance level (p <0.001), suggesting that the relationship between this factor and the detection of methamphetamine followed a curved line. In addition, five interaction terms were found significant in their respective coefficients which suggested their relation-

ships between the two factors. The contour plots of these interaction terms are shown in Figure 3.

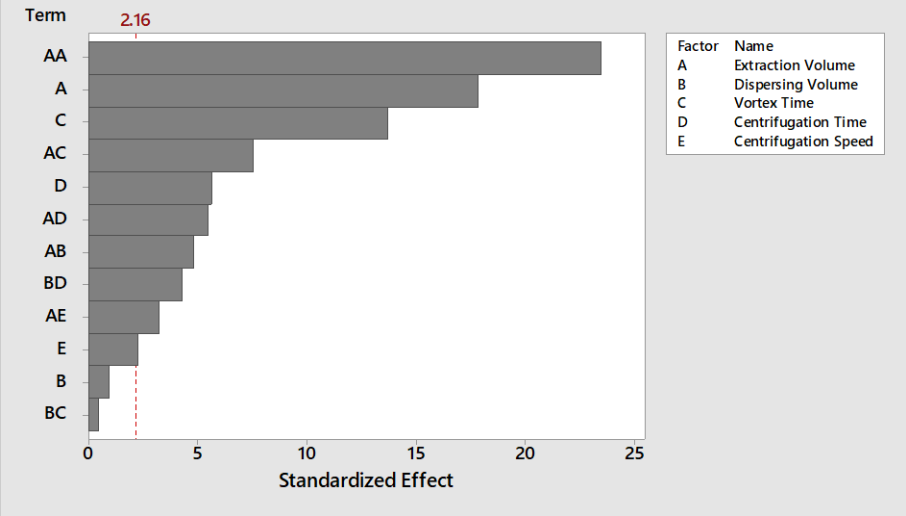

Figure 2. Pareto chart of the standardized effects

Table 2. ANOVA of the regression coefficient for the recovery of methamphetamine area

Source	DF	Adj SS	Adj MS	F-value	*p* -value
Model	12	226.054	18.8378	182.31	<0.001
Linear	5	82.938	16.5877	160.53	<0.001
x_1	1	32.975	32.9752	319.13	<0.001
x_2	1	0.087	0.0870	0.84	0.375
x_3	1	19.417	19.4174	187.92	<0.001
x_4	1	3.309	3.3085	32.02	<0.001
x_5	1	0.529	0.5289	5.12	0.041
Square	1	56.941	56.9406	551.06	<0.001
x_1*x_1	1	56.941	56.9406	551.06	<0.001
2-way interaction	6	48.088	8.0147	77.57	<0.001
x_1*x_2	1	2.426	2.4258	23.48	<0.001
x_1*x_3	1	5.885	5.8852	56.96	<0.001
x_1*x_4	1	3.119	3.1188	30.18	<0.001
x_1*x_5	1	1.099	1.0988	10.63	0.006
x_2*x_3	1	0.022	0.0223	0.22	0.650
x_2*x_4	1	1.894	1.8939	18.33	<0.001
Error	13	1.343	0.1033		
Total	25	227.397			

DF= Degree of freedom; Adj SS=Adjusted sum of squares; Adj MS=Adjusted Mean sum of squares

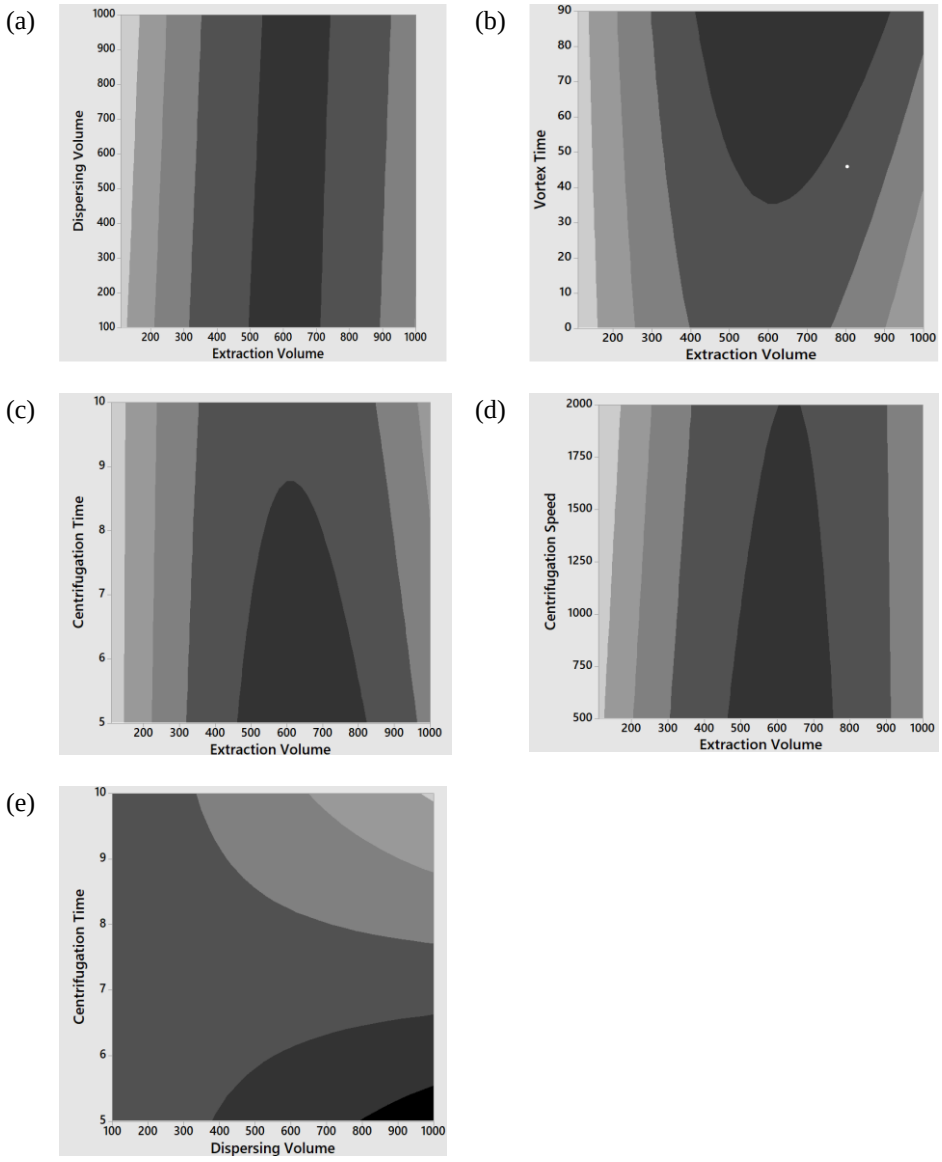

Figure 3. Contour plots showing interaction between (a) volume of extraction solvent vs volume of dispersive solvent (p<0.001); (b) volume of extraction solvent vs duration of vortex agitation (p<0.001); (c) volume of extraction solvent vs centrifugation time (p<0.001); (d) volume of extraction solvent vs centrifugation speed (p= 0.006); and (e) volume of dispersive solvent vs centrifugation time (p < 0.001)

Interaction between volume of extraction solvent and four variables (i.e. volume of dispersive solvent, duration of vortex agitation, centrifugation time, and centrifugation speed) were significant as shown in Figure 3(a)-(d). The darker regions in each figure indicated the higher extraction efficiency for methamphetamine. Based on Figure 3(a), the extraction efficiency increased with the increase of the volume of extraction solvent until certain point at approximately 700 µL and get reduced afterwards. Note also that a longer duration of vortex agitation, optimized at 90 s [Figure 3(b)], followed by a slower centrifugation time for 5 mins and shorter and centrifugation speed at 500 rpm, respectively [Figure 3(c)-(d)] were found to have

increased the extraction efficiency. The interaction between dispersive volume and centrifugation time was also highly significant [Figure 3(e)]. It was evident that with an increase in the dispersive volume up to a volume of 1000 µL and a decrease in centrifugation time to 5 mins, respectively, the efficiency of methamphetamine extraction was raised significantly.

Using the DLLME optimized conditions which shown in Table 3, a mixture containing 685 µL dichloromethane (extraction solvent) and 1000 µL 2-propanol (dispersive solvent) was injected into the sample solution. The ternary system was subjected to vortex agitation for 90 seconds, followed by centrifugation at 500 rpm for 5 mins. Note that the volume of extraction solvent was optimized to 685 µL, where this is the point indicating that when the extraction volume was too low, the extractant amount was very small and therefore unable to extract the analyte efficiently. On the contrary, the analyte would become diluted with high volume of extraction solvent, minimizing the amount of target substance to be extracted [34]. It was also observed that a longer duration of vortex agitation could aid in handling the complex matrices of the extraction, where a shorter period of centrifugation time could achieve the full separation of emulsion [35]. The equilibrium state could be immediately achieved after the addition of solvent and vortex agitation of the sample solution; therefore, a short centrifugation duration and speed were found sufficient to achieve a high extraction efficiency [36].

Table 3. Multiple response prediction for the response of methamphetamine detection.

Factor	Optimized Settings
Volume of extraction solvent	685 µL
Volume of dispersive solvent	1000 µL
Duration of vortex agitation	90 s
Centrifugation time	5 min
Centrifugation speed	500 rpm

Recovery of methamphetamine from laboratory coat materials

Laboratory coat material samples deposited with defined masses of methamphetamine (0.5 µg, 1,5 µg and 3.0 µg) were treated with proposed DLLME protocol, derivatized with TFAA, and subjected to GC-MS analysis. Figure 4 shows the mean percentages of recovery with seven type of laboratory coat materials. On average, at least 45% of methamphetamine were successfully recovered from the laboratory coat materials, depending on the concentration levels and types of fabric materials. Previously, solvent extraction [37] and liquid-liquid extraction (LLE) [38] were also used to extract methamphetamine from fabric materials followed by its detection through instrumental techniques. When compared to these conventional sample extraction methods, our DLLME procedure is less laborious while avoiding the drawbacks suffered from the conventional LLE, such as the need for excessive quantities of chemicals, reagents and sample, slow and long extraction time, and the formation of emulsions.

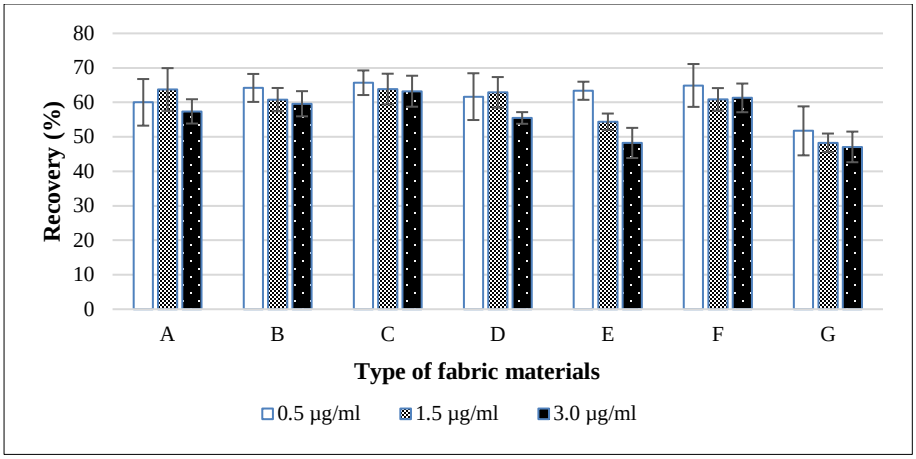

Figure 4. Mean percentages of recovery for methamphetamine from seven type of laboratory coat materials

Extraction efficiency of methamphetamine from the laboratory coat materials could be impacted by the physio-chemical interactions of solute of drugs with the surfaces and their penetration or diffusion into porous substrates [39]. It is also worth noting that the penetration is a complex phenomenon involving the interaction between solvent and porous substrates, depending on surface tension and the contact angle between each other [40]. As compared to studies which emphasized on the extraction of the drug substance from non-porous surfaces, the extraction efficiencies were also reported to be varied based on the surfaces, where only approximately 60% of the methamphetamine could be recovered from the varnished wood substrate [27].

Our study showed slightly variation in term of the amount of methamphetamine which successfully recovered from different laboratory coat materials through the application of proposed extraction protocol. In general, the %RSD reported was in an acceptable range, falling between 3.14-9.90%. Sorption of methamphetamine compounds on a fabric could have been influenced by its physical properties of the fibers, including its porosity [41]. Fabric materials with polar and slightly basic in nature, methamphetamine might also have great affinity for cotton that made up of polar cellulose, waxes, proteins, and fatty acids [42]. These factors certainly deserve further studies.

Although the DLLME procedure was found to be effective in extracting methamphetamine from the laboratory coat materials. As compared to other extraction techniques such as conventional LLE, DLLME procedure can be completed in a relatively quicker manner, consuming low volume of solvent, and requiring shorter extraction time. It is cost effective and environmentally friendly, and therefore widely applied in trace analysis [43]. DLLME also aids in minimizing the tendency of forming emulsion as frequently encountered during LLE procedure [44]. Based on our findings, it can be predicted that there is at least two-fold of drugs actually present on the laboratory coat materials than those detected by the protocol here. The laboratory monitoring program should therefore take corrective actions if recovered concentration exceeds the safe exposure level. As a future recommendation, the composition and characteristics of laboratory coat materials should be further explored to provide details insight on the influential factors that might affect the recovery of analytes.

It was noted that the proposed method was destructive in nature if the fabric material has to be cut out for extraction. Bitter [39] had proposed a wiping procedure in which a surface contaminated with methamphetamine was wiped and analyzed; however, the methamphetamine was detected at a very low amount with low recoveries, indicating the extraction might not adequately efficient to recover the target substance from porous samples. To apply the proposed method in the operational settings, similar fabric material can be

patched onto a laboratory coat and detached for extraction and analysis during routine monitoring to avoid destruction of the laboratory coats. The protocol of methamphetamine deposition used here was adapted from studies on household surfaces, and shall also be explored in future studies, especially on the contamination mechanisms and how they affect the recoveries of target substances. In general, this study has successfully demonstrated the possibility of determining methamphetamine as an indicator of clothing contamination from the fabrics, where it would benefit the screening of methamphetamine contamination on laboratory coats, and subsequently in planning the appropriate corrective or preventive actions and recommendations to address the contamination issues.

Conclusion

Determination of methamphetamine from laboratory coat materials was successfully carried out using a PBD optimized DLLME procedure coupled with GC-MS analysis. An addition of a combination of 685 µL dichloromethane (extraction solvent) and 1000 µL 2-propanol (dispersive solvent) into the sample solution followed by a 90s vortex agitation and a centrifugation at 500 rpm for 5 mins produced the highest extraction efficiency for methamphetamine. Based on the proposed procedure, at least 45% of methamphetamine could also be successfully recovered from the fabric materials. Repeatable recoveries of the drug substance were achieved from the laboratory coat materials, varied on their respective composition. As future recommendation, the properties of fabric materials that could influence the recovery of methamphetamine shall be investigated, in addition to the exploration on the contamination mechanisms of drug substances on these fabric materials.

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References

1. Sisco, E., Najarro, M. and Burns, A. (2018). A snapshot of drug background levels on surfaces in a forensic laboratory. *Forensic Chemistry*, 11: 47-57.
2. Broadwater, K. R., Jackson, D. A. and Li, J. F. (2020). Evaluation of occupational exposures to illicit drugs at controlled substances laboratories. Health Hazard Evaluation Report 2018-0090-3366. *Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health*.
3. Sisco, E. and Najarro, M. (2019). Multi-laboratory investigation of drug background levels. *Forensic Chemistry*, 16: 100184.
4. Wright, J., Walker, G. S. and Ross, K. E. (2019). Contamination of homes with methamphetamine: Is wipe sampling adequate to determine risk? *International Journal Environmental Research Public Health*, 16(19): 3568.
5. United Nations Office on Drugs and Crime. (2021). Global overview: Drug demand and drug supply. World Drug Report 2021. *Vienna, Austria: United Nations*.
6. Daughton, C. G. (2010). Illicit drugs and the environment. In: Castiglioni S, Zuccato E, Fanelli R. (Editors). Illicit Drugs in the Environment, LA: John Wiley & Sons, Inc.
7. Kairigo, P., Ngumba, E., Sundberg, L. R., Gachanja, A. and Tuhkanen, T. (2020). Contamination of surface water and river sediments by antibiotic and antiretroviral drug cocktails in low and middle-income countries: Occurrence, risk and mitigation strategies. *Water*, 12(5): 1376.
8. Lin, Y. C, Hsiao, T. C. and Lin, A-YC. (2020). Urban wastewater treatment plants as a potential source of ketamine and methamphetamine emissions to air. *Water Research*, 172: 115495.
9. Jenkins, A. J. (2001). Drug contamination of US paper currency. *Forensic Science International,* 121: 189-193.
10. Smith, F. P. and McGrath, K. R. (2011). Cocaine surface contamination and the medico-legal implications of its transfer. *Egyptian Journal Forensic Sciences*, (1): 1-4.
11. Mastroianni, N., Postigo, C., López de Alda, M., Viana, M., Rodríguez, A., Alastuey, A. Querol, X. and Barceló, D. (2015). Comprehensive monitoring of the occurrence of 22 drugs of abuse and transformation products in airborne particulate

matter in the city of Barcelona. *Science of Total Environment*, 532: 344-352.

12. Nair, M. V. and Miskelly, G. M. (2019). Determination of airborne methamphetamine via capillary microextraction of volatiles (CMV) with on-sorbent derivatisation using o-pentafluorobenzyl chloroformate. *Forensic Chemistry*, 14:100161.
13. Chen, X., Wu, X., Luan, T., Jiang, R. and Ouyang, G. (2021). Sample preparation and instrumental methods for illicit drugs in environmental and biological samples: A review. *Journal of Chromatography A*, 1640: 461961.
14. Kuhn, E. J., Walker, G. S., Whiley, H., Wright, J. and Ross, K. E. (2019). Household contamination with methamphetamine: Knowledge and uncertainties. *International Journal of Environmental Research and Public Health*, 16(23): 4676.
15. Martyny, J. W., Arbuckle, S. L., McCammon, C. S., Esswein, E. J., Erb, N. and Van Dyke, M. (2007). Chemical concentrations and contamination associated with clandestine methamphetamine laboratories. *Journal of Chemical Health and Safety*, 14(4): 40-52.
16. Doran, G. S., Deans, R. M., Filippis, C. D., Kostakis, C. and Howitt, J. A. (2017). The presence of licit and illicit drugs in police stations and their implications for workplace drug testing. *Forensic Science International*, 278: 125-136.
17. Yeh, K., Li, L., Wania, F. and Abbatt, J. P. (2022). Thirdhand smoke from tobacco, e-cigarettes, cannabis, methamphetamine and cocaine: Partitioning, reactive fate, and human exposure in indoor environments. *Environment International*, 160: 107063.
18. Kerry, G. L., Ross, K. E., Wright, J. L. and Walker, G. S. (2022). A review of methods used to detect methamphetamine from indoor air and textiles in confined spaces. *Toxics*, 10(11): 710.
19. Doran, G. S., Deans, R. M., Filippis, C. D., Kostakis, C. and Howitt, J. A. (2017). Quantification of licit and illicit drugs on typical police station work surfaces using LC-MS/MS. *Analytical Methods,* 9:198-210.
20. United Nations Office on Drugs and Crime. (2020). Synthetic Drugs in East and Southeast Asia: Latest developments and challenges. *Vienna, Austria: United Nations*.
21. Teoh, W. K., Mohamed Sadiq, N. S., Saisahas, K., Phoncai, A., Kunalan, V., Md Muslim, N. Z., Limbut, W., Chang, K. H. and Abdullah, A. F. L. (2022). Vortex-assisted dispersive liquid–liquid microextraction-gas chromatography (VADLLME-GC) determination of residual ketamine, nimetazepam, and xylazine from drug-spiked beverages appearing in liquid, droplet, and dry forms. *Journal of Forensic Sciences,* 67(5): 1836-1845.
22. Lemos, V. A., Barreto, J. A., Santos, L. B., de Assis, R. dos S., Novaes, C. G. and Cassella, R. J. (2022). In-syringe dispersive liquid-liquid microextraction. *Talanta*, 238:123002.
23. Jain, R., Jain, B., Chauhan, V., Deswal, B., Kaur, S., Sharma, S. and Abourehab, M. A. (2023). Simple determination of dichlorvos in cases of fatal intoxication by gas chromatography-mass spectrometry. *Journal of Chromatography B*, 1215: 123582.
24. Meng, L., Zhang, W., Meng, P., Zhu, B. And Zheng, K. (2015). Comparison of hollow fiber liquid-phase microextraction and ultrasound-assisted low-density solvent dispersive liquid-liquid microextraction for the determination of drugs of abuse in biological samples by gas chromatography-mass spectrometry. *Journal of Chromatography B*: *Analytical Technologies in the Biomedical and Life Sciences,* 989: 46-53.
25. Manwar, J., Mahadik, K. and Paradkar, A. (2013). Plackett–Burman design: A statistical method for the optimization of fermentation process for the yeast Saccharomyces cerevisiae isolated from the flowers of *Woodfordia fruticosa. Fermentation Technology*, *2*: 109.
26. Abd El-Hamid, H. T., Al-Prol, A. E. and Hafiz, M. A. (2018). Plackett-Burman and response surface methodology for optimization of oily wastewater bioremediation by Aspergillus sp. *South Asian Journal of Research in Microbiology*, 2018: 1-9.
27. Abdullah, A. F. L. and Miskelly, G. M. (2010). Recoveries of trace pseudoephedrine and methamphetamine residues from impermeable

household surfaces: Implications for sampling methods used during remediation of clandestine methamphetamine laboratories. *Talanta.* 81(1-2): 455-461.

28. Quigley, A., Cummins, W. and Connolly, D. (2016). Dispersive liquid-liquid microextraction in the analysis of milk and dairy products: A review. *Journal of Chemistry*, 2016: 4040165.
29. Saraji, M. And Boroujeni, M. K. (2014). Recent developments in dispersive liquid–liquid microextraction. *Analytical and Bioanalytical Chemistry*, 406: 2027-2066.
30. Salim, S. A., Sukor, R., Ismail, M. N. and Selamat, J. (2021). Dispersive liquid–liquid microextraction (DLLME) and LC-MS/MS analysis for multi-mycotoxin in rice bran: method development, optimization and validation. *Toxins*, 13(4): 280.
31. Tan, Y. H., Chai, M. K. and Wong, L. S. (2018). A review on extraction solvents in the dispersive liquid-liquid microextraction. *Malaysian Journal of Analytical Sciences*, 22(2): 166-174.
32. Al-Saidi, H. M. and Emara, A. A. A. (2014). The recent developments in dispersive liquid-liquid microextraction for preconcentration and determination of inorganic analytes. *Journal of Saudi Chemical Society*, 18(6): 745-761.
33. Geiger, E. O. (2014). Statistical methods for fermentation optimization. In: Todaro CC, Vogel HC, editors. Fermentation and biochemical engineering handbook. NY: William Andrew.
34. Rahmani, M., Ghasemi, E. and Sasani, M. (2017). Application of response surface methodology for air assisted-dispersive liquid- liquid microextraction of deoxynivalenol in rice samples prior to HPLC-DAD analysis and comparison with solid phase extraction cleanup. *Talanta.* 165: 27-32.
35. Guo, L. and Lee, H. K. (2013). Vortex-assisted micro-solid-phase extraction followed by low-density solvent based dispersive liquid-liquid microextraction for the fast and efficient determination of phthalate esters in river water samples. *Journal of Chromatography A,* 1300: 24-30.
36. Sanagi, M. M., Mokhtar, S. U., Ibrahim, W. A. W. and Aboul-Enein, H. Y. (2013). Application of dispersive liquid-liquid microextraction based on solidification of floating organic droplet to the analysis of antidepressant drugs in water samples. *Sains Malaysiana,* 42(2): 1490-157.
37. Al-Dirbashi, O. Y., Ikeda, K., Takahashi, M., Kuroda, N., Ikeda, S. and Nakashima, K. (2001). Drugs of abuse in a non-conventional sample; detection of methamphetamine and its main metabolite, amphetamine in abusers' clothes by HPLC with UV and fluorescence detection. *Biomedical Chromatography*, 15: 457-463.
38. Keasey, S. J. (2011). Testing for the presence of methamphetamine residues on clothing from suspected clandestine labs. Master's Thesis, AL: University of Alabama at Birmingham.
39. Bitter, J. L. (2017). The persistence of illicit drug smoke residues and their recovery from common household surfaces. *Drug Testing and Analysis*, 9(4): 603-612.
40. Lee, S. and Obendorf, S. K. (2001). A statistical model to predict pesticide penetration through nonwoven chemical protective fabrics. *Textile Research Journal*, 71(11): 1000-1009.
41. Saini, A., Okeme, J. O., Mark Parnis, J., McQueen, R. H. and Diamond, M. L. (2017). From air to clothing: characterizing the accumulation of semi-volatile organic compounds to fabrics in indoor environments. *Indoor Air,* 27(3): 631-641.
42. Morrison, G., Shakila, N. V. and Parker, K. (2015). Accumulation of gas-phase methamphetamine on clothing, toy fabrics, and skin oil. *Indoor Air,* 25(4): 405-414.
43. Li, M. J., Zhang, H. Y., Liu, X. Z., Cui, C. Y. and Shi, Z. H. (2015). Progress of extraction solvent dispersion strategies for dispersive liquid-liquid microextraction. *Chinese Journal of Analytical Chemistry*, 43(8):1231-1240.
44. Majors, R. E. (2013). Sample preparation fundamentals to chromatography. Canada: Agilent Technologies.