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Contents

Forensic Classification of Glass Employing Refractive Index Measurement

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ABSTRACT: Burglary and accident cases may involve glass fragments as physical evidence found at the crime scene. In forensic investigation, the major physical examination to determine the origin of glass is refractive index (RI) measurement. It was therefore of interest to determine RI measurements of several types of glasses commonly found in Malaysia with a view of classifying glass as building and automobile glasses. Twenty samples of glass from each classification were collected from car workshops and glass pane shops. Determination of RI value was affected using Glass Refractive Index Measurement 3 (GRIM3) instrument. From this study, the RI values of automobile glass can be classified into 3 types according to their RI values and thickness. Windscreen glass was found to be in the RI range of 1.5152 – 1.5225, rear screen glass with RI of 1.5147- 1.5217 and side window glass with RI range of 1.5188-1.5190, all samples with thickness of between 2 – 6 mm. Building glass can be classified into heat absorbing float (1.5197 – 1. 5211), clear float (1.5189 – 1.5213), figured float (1.5164 – 1.5234) and reflective float (1.5167 - 1.5188) with sample thicknesses of 2 – 6 mm. The results show that each glass type has different range of RI value which is related to thickness, manufacturer and colour due to its end-use. Thus, the origin of glass according to its end-use types could be determined by the relationship between RI and thickness to assist forensic scientists in their investigation.

Keywords: Refractive Index (RI), Glass fragment, glass type, GRIM3

Introduction

Glass fragments constitute as contact trace evidence that are often sent to forensic laboratory for examination especially in cases involving accidents or house breaking [1]. The fragment size is often equal to or less than 1 mm [2].

One of the problems in forensic glass analysis is comparison between known and unknown glass to establish their origin or to aid in sample matching purposes [3]. In addition, determination of glass classification regarding end-use type categories is a difficult task in forensic glass analysis. It is important especially when there is no control sample found at the crime scene for the comparison process [4]. Therefore knowledge on the type of glass may help forensic scientist in forensic glass investigation.

Refractive index (RI) measurement is the most common method employed for glass analysis [5-7]. Refractive index (RI) value was reported to be dependent upon on the nature of raw materials used and manufacturing process especially during annealing process [8, 9]. Distribution of RI was slightly affected by types of glass according to its color, manufacturer and thickness [10].

Safety window glass is one of the items found as safety features in an automobile. There are two main types of safety glass used in automobile today which are laminated glass and tempered glass [11]. Laminated glass that consists of a layer of plastic between two glass panes is commonly used as windscreen. Tempered glass is used as side and rear windows. Building glass comprised of various types of float glass that are used as window, door and partition. Examples of common types of float glass are clear glass, tinted (heat-absorbing) glass, reflective glass and low-emissivity glass [11].

In this study, glass fragments are classified according to end-user type using RI measurements and glass thickness.

Experimental

i. Sampling

Freshly broken glass pieces collected from car workshops in Kajang, Selangor, Malaysia with 14 samples of windscreen, three samples of rear screen and three samples of side window glasses were obtained.

Four samples of clear float glass, four samples of heat absorbing float glass, four samples of reflective float glass and eight samples of figured float glass as building glass were taken from glass pane shops in Bandar Baru Bangi, Selangor, Malaysia. These glasses were from several manufacturers, colors and thicknesses.

ii. Refractive Index Measurement

RI of glass was determined by oil immersion method using GRIM3 system (Foster & Freeman). Selected glass fragments were mounted onto glass slides and immersed in silicon oil B. Each prepared slide was inserted into a hot stage (Mettler Toledo FP82HT) and illuminated using monochromatic sodium light at 589 nm. Temperature of the hot stage was controlled by the GRIM3 unit equipped with an attached video camera and a phase contrast microscope (LEICA DM 2500).

The observation in measuring RI was noted by the disappearance of the Becke line and minimum contrast between the glass and liquid medium. The temperature at which the Becke line disappeared was taken as the match point, at which the RI of the glass fragment equals to the RI of oil at that temperature.

Results and Discussion

Automobile glass is commonly used as windscreen, rear screen and side window of a vehicle. The relationship of RI values with glass thickness for automobile glass is shown in Figure 1. Three clusters were obtained corresponding to the types of vehicle glass. RI values for windscreen glass was in the range of 1.5152 – 1.5225 for glass thickness of between 2-3 mm. Most laminated windscreen glass consisted of 2 glass colors, with green as the outer glass layer and a transparent inner glass layer.

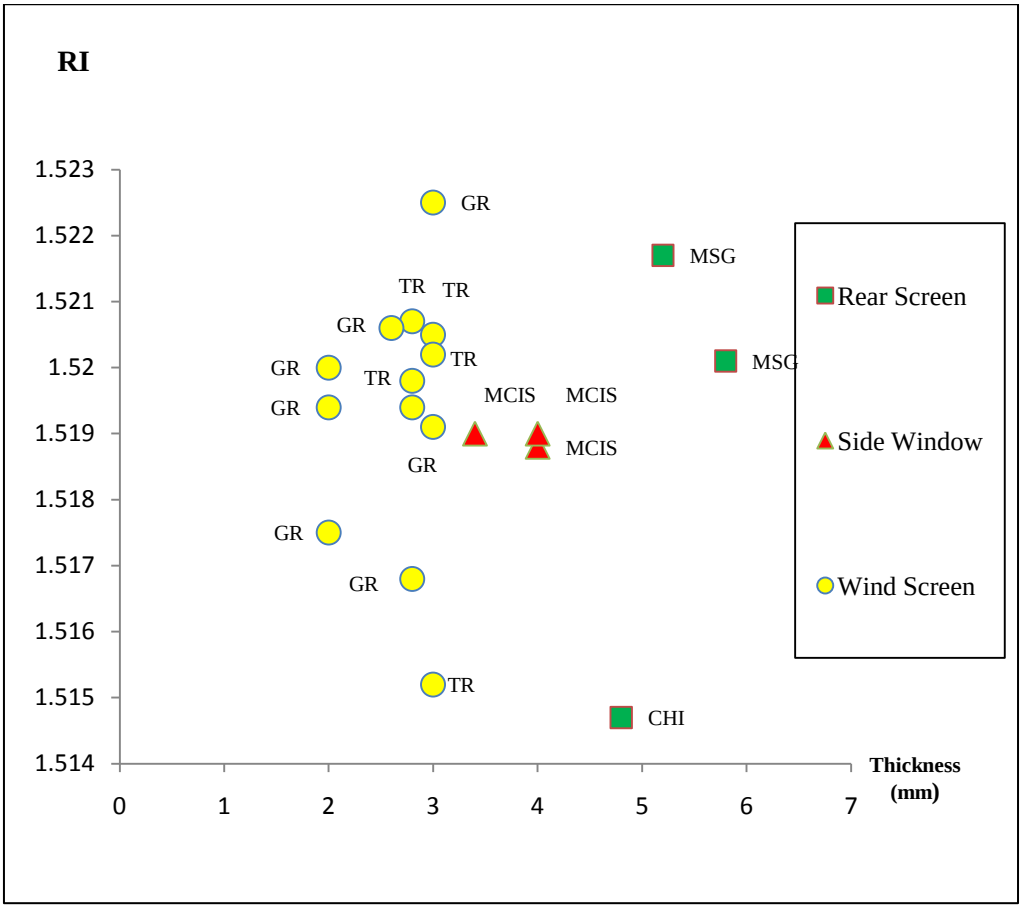

Fig. 1: Graph of RI value verse thickness for automobile glass, where MSG denotes Malaysian Sheet Glass, CHI denotes China, MCIS denotes Malaysia Cooperative Insurance Society Safety Glass, TR as Transparent and GR as Green.

Three side window samples (manufactured by MCIS) with thickness of 3.0 – 4.0 mm show close RI values of 1.5190 and 1.5188. Two fragments that display the same RI of 1.5190 were found to originate from the domestic manufactured car (Proton Persona and Proton Exora). Rear window glass with thickness of more than 4 mm showed the RI values in a wide range from 1.5147 to 1.5217. This is mainly attributed to the different glass manufacturer (from China and Malaysian Sheet Glass, MSG).

Graph of RI versus thickness for building glass, shows four clusters corresponding to the four types of building glass (Figure 2). Heat absorbing float glass with thickness between five and six mm, had the RI values of 1.5197 – 1.5211. Note that the colour of glass has a profound effect on the RI value measured. For instance, green glass was found to have higher RI (1.5210 and 1.5211) compared to grey glass (1.5197 and 1.5205) for this type of building glass.

RI for figured float glass was found to be distributed between 1.5164 and 1.5234 with thickness between 2.8 and 4.2 mm. The large RI variation is mainly attributed to its colour. The highest RI value measured (1.5234) was from the green colour glass. This was followed by the grey glass (1.5220), the transparent glass (1.5196 – 1.5205) while the lowest RI was displayed by blue r glass (1.5164).
Clear float glass has two ranges of thickness which are two mm and 5-6 mm, respectively. Both thickness shared the same range of RI (1.5189 – 1.5213). Since all samples were colourless, variation of RI was mainly due to different manufacturer of glass samples. Samples with thicknesses of 5-6 mm were from Malaysian Sheet Glass (MSG) and Kein Safety Glass (KSG). A coloured float glass manufactured by Hesin Glass gave a lower RI value.

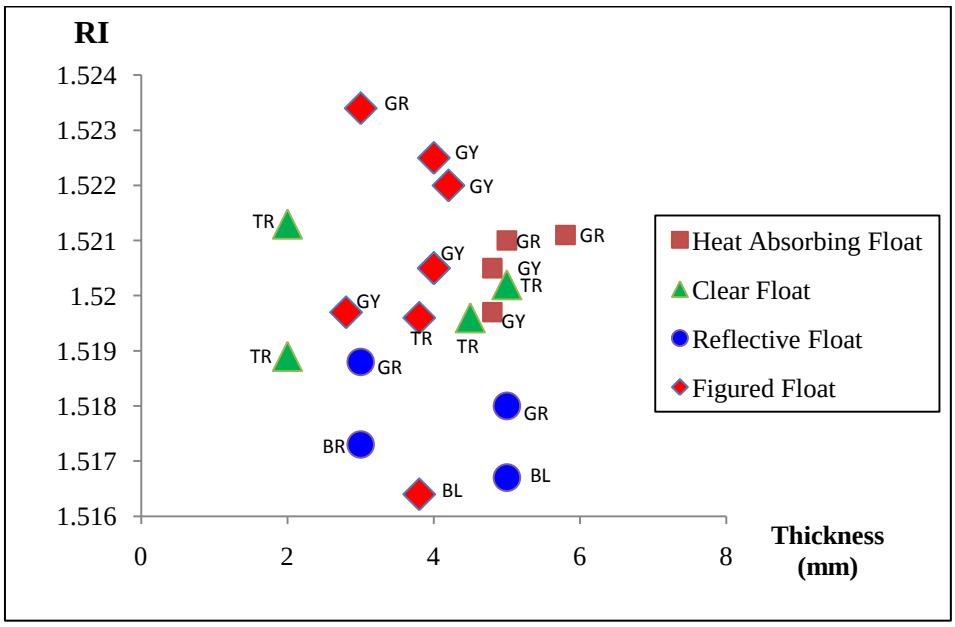

Fig. 3: Graph of RI value verse thickness for building glass where GR denotes green, GY denotes gray, TR denotes transparent, BR denotes bronze and BL denotes blue color.

The results show that reflective float glass has lower RI value than other types of glass (1.5167 - 1.5188) with thickness between 3 – 5 mm. Since all the samples came from same manufacturer (MSG), the variation of RI values are attributed to the different colors of glass (green, bronze and blue).

RI of building glass is highly affected by the colour of the glass. Nevertheless, the type of glass due to its intended end-use can be classified via its relationship between refractive indices and thicknesses. The study revealed that each type of building glass has high RI for green colored float glass and lowest RI for blue colored glass from the same manufacturer.

Conclusion

Classification of automobile and building glasses by their end-use type has been performed using refractive index measurements. Automobile glass can be classified into 3 types according to their RI values and thickness. Windscreen glass was found to be in the RI range of 1.5152 – 1.5225, rear screen glass with RI of 1.5147- 1.5217 and side window glass with RI range of 1.5188-1.5190, for all samples with thicknesses of between 2 – 6 mm. On the other hand, building glass can be classified into 4 end-user types; namely heat absorbing float (1.5197 – 1. 5211), clear float (1.5189 – 1.5213), figured float (1.5164 – 1.5234) and reflective float (1.5167 - 1.5188) with sample thicknesses of 2 – 6 mm.

Each glass type has different range of RI value which is related to thickness, manufacturer and color according to the end-use of glass. Thus, the origin of glass according to its end-use types could be determined by the relationship between RI and thickness to assist forensic scientists in their glass examination.

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Application of Multivariate Chemometry for Discrimination of Black Ballpoint Pen Inks Based on the IR Spectrum

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Abstract: This preliminary work investigates whether FTIR, in combination with multivariate analysis can be used to differentiate 24 black ballpoint pen inks from six different varieties. Ink entries from black ballpoint pens were analyzed directly on paper using micro ATR-FTIR spectroscopy technique and the data obtained was processed and evaluated by a series of multivariate chemometrics. Absorbance value from wavenumbers of 2000-675 cm^{-1} were first grouped into eight different clusters by cluster analysis (CA), followed by principal component analysis (PCA) to form a set of new variables. After that, discriminant analysis (DA) and one-way ANOVA were conducted using the new variables set. Results showed that six black ballpoint pen varieties could be classified into three main groups via discriminant analysis (DA). Differentiation analyses of six different pen varieties performed using one-way ANOVA indicated only two pairs of varieties cannot be differentiated at 95% confidence interval. It can be concluded that micro-ATR FTIR spectroscopy coupled with chemometric techniques could make a powerful non-destructive discriminating tool for analysis of inks based on their infrared spectrum.

Keywords: ATR-FTIR spectroscopy; ink analysis; multivariate analysis; discriminant analysis

Introduction

In general, forensic document examination comprised of handwriting identification and ink analysis. Ink analysis aims at revealing information of chemical components inside inks. It can be of destructive or non-destructive in nature. Nowadays, most of the techniques used by forensic ink analyst posses some disadvantages. In brief, ink analysis techniques available nowadays possed three main weaknesses. Firstly, techniques like high performance liquid chromatography (HPLC) and gas-chromatography mass spectrometry (GCMS) can give better results in term of resolution and also able to detect more components of inks [1-3]. However, those techniques are destructive where part of the sample need to be destroyed or dissolved to prepare the sample in the form that suitable to be analysed by aforementioned techniques. During court procedure, integrity of evidence is the main issue to be discussed. Secondly, if the quantity of the available sample is too little, desctructive techniques cannot be applied on it. Thirdly, non-destructive techniques such as Raman and FTIR spectroscopy usually do not give much information about inks and the interpretation of the spectrum tended to be biased as it depends on human being eye to do the evaluation [4, 5].

As such, this study has been conducted to develop an ink analysis method that is able to give result comparable to destructive techniques while ensuring the integrity of sample. In this study, feasibility of ATR-FTIR spectroscopy coupled with multivariate techniques for ink analysis has been explored. The chief advantages of micro-ATR FTIR spectroscopy are that it is non-destructive, does not require any prior preparation of the sample, requires only a small amount of sample for examination, not time-consuming due to its short analysis time, eco-friendly and cost-effective as it does not involve any reagents.

On the other hand, multivariate analysis is useful in handling high dimensional spectra data and in assessing quantitatively the minor differences of a particular ink relative to other inks that otherwise could not be noticed easily by visual inspection. In this study, principal component analysis (PCA) and discriminant analysis (DA) have been used to process the data. PCA is an unsupervised multivariate statistical method which reduced a large number of raw data to a smaller number of principal components. Each of the principal components formed is a linear combination of the raw variables that represents significant variability in the data but which are uncorrelated with each other [1]. Here, the raw variables refer to the list of wavenumbers. Whereas, DA is a supervised multivariate statistical

method that specifically attempts to describe, model and explain the differences between known classes (category variable) based on list of response variables [6]. In this study, the six different varieties of pens act as category variables and response variables would be the newly formed list of principal components.

Experimental

Samples

A total of 24 black ballpoint pens of six different varieties were purchased from a stationary shop at Subang Jaya, Selangor, Malaysia. All of these pens were obtained in multiple packs of the same product to ensure all of them were from the same production batches as the variation between batches will not be considered in this study. Each pen variety was allocated a sample ID for the purpose of this study. Detailed descriptions of collected samples used in this study are presented in Table 1. Double A™, (Thailand) was the white paper used throughout the study. Each of the 24 pens was used to draw three different small circles with a 3 mm diameter on a piece of paper. Experimental work was carried out on the ink deposited on the white paper not later than one day after drawing to minimize changes due to ink aging.

Micro-ATR-FTIR Analysis

All experimental spectroscopy was carried out on a Thermo Scientific Nicolet iN10 MX FT-IR microscope with mercury cadmium telluride (MCT) detector. A *Ge* crystal tip ATR objective was used as micro-sampling accessory. The background spectrum was reacquired after every analysis to reduce variation in the spectra due to instrumental drift. Each spectrum was the result of an average 16 scans at 4 cm^{-1} resolution over a spectral range of 4000 to 675 wavenumbers (cm^{-1}). Three spectrums were collected from each of the 24 pens. The IR spectral data was stored in a data matrix in Microsoft Excel® spreadsheet.

Software

Data collected was processed and analyzed using statistical package SPSS (Statistical Package for the Social Sciences, Windows version 15.0, SPSS Inc., Chicago, USA).

Normalization of Selected IR Region

All three spectra of each sample were included to assess the material reproducibility and homogeneity while ensuring representativeness of each sample due to the minimal size of inks contacted by ATR *Ge* tip and the potential heterogeneity of the inks [7]. Triplicate analysis of 24 pens gave 72 spectra. As such, full data set comprised 72 spectra, consisting of absorbance values as a function of wavenumber, were normalized prior to carrying out the multivariate analysis to reduce variation in the data due to different thickness of the pen inks deposited on paper samples. The absorbance values for each spectrum were divided by the total absorbance resulting from each spectrum across wavenumbers 2000-675 cm^{-1}.

Transformation of Raw Data to New Set of Variables

In this study, the selected IR region (2000-675 cm^{-1}) composed of 688 raw data. The normalized absorbance values were transformed into a new set of variables. This is an important step as discriminant analysis (DA) is restrictive with regard to the number of predictor variables versus the number of samples. In addition, a smaller number of input variables also means results can be obtained with reduced computational and economical expense while improving the accuracy and efficiency of the classification tasks [8]. Variable reduction was achieved by conducting cluster analysis and principal component analysis sequentially. Initially, cluster analysis was performed on the raw variables (688 data points) over the objects (ink samples) by using Ward's method and squared Euclidean distance to form eight different clusters. Each cluster shall contain the same variables describing the ink composition variables that carry similar information about the objects. Subsequently, correlation PCA was performed on each of the eight clusters, separately. The number of components to be extracted was decided based on the Kaiser criterion [9]. The new set of variables comprised all selected principal components that were labeled accordingly, e.g. PCA 1_2 refers to the first component produced by PCA conducted on the second cluster of data points.

Results & Discussion

Infrared Spectrum

Although all the spectra were scanned from 4000-675 cm^{-1}, only the regions from 2000-675 cm^{-1} were analyzed. The IR absorption peaks at the region between 4000 and 2000 cm^{-1} and this was mainly due to water vapour and carbon dioxide from the atmosphere and only a few weak and broad bands attributable to ink [8]. The respective spectra of six different varieties of black ballpoint pen inks analyzed by micro-ATR-FTIR spectroscopy in the region of 2000-675 cm^{-1} are shown in Figure 1.

Table 1: Sample identification for all six studied black ballpoint pen varieties

Sample ID	Sample Name	Quantity
19	G-soft gs 66	3
20	G-soft gs 77	3
21	G-soft r 100	4
22	MGM BP 713	6
23	MGM e-Rite	4
24	PILOT Super GP	4

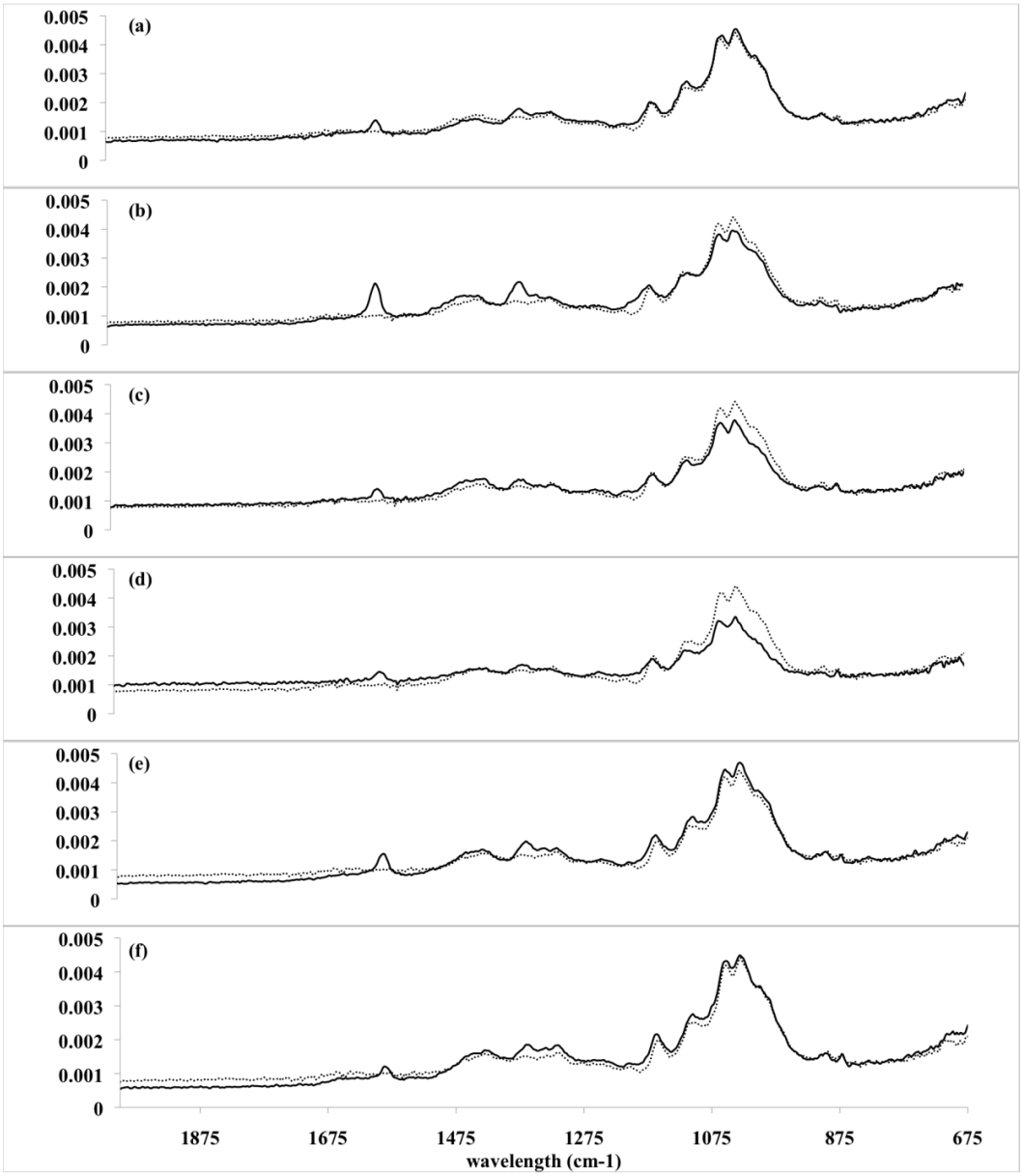

Figure 1: The respective spectra of six black ballpoint pen varieties (–) and blank paper (…) analyzed by micro-ATR-FTIR spectroscopy in the region of 2000-675 cm^{-1}: (a)19; (b) 20; (c) 21; (d) 22; (e) 23; and (f) 24.

In general, all spectra from six different varieties were showing similar spectral patterns. All exhibit a prominent peak at approximately 1584 cm^{-1} but with differences in the form of relative peak height and its

shape (Figure. 1) in which 19, 21, 22 and 24 gave lowest absorption intensity while 20 gave the highest absorption intensity. Besides, 20 also showed few minor peaks at region between 1500-1400 cm^{-1}. Peak at 1584 cm^{-1} corresponding to a skeletal vibration of triarylmethane dye and the C=C stretch vibration of epoxy resin (about 1581 cm^{-1}) [10]. As such, the quantity of crystal violet may play an important role in the differentiation of those six pen models. The region between wavenumbers 1100-1000 cm^{-1} is of less concern as it contains peaks mostly from paper substrate [11]. The vibrational assignment for other peaks has been explained elsewhere [10].

Discriminant Analysis (DA)

Stepwise DA was conducted on the new set of variables to see whether all 72 IR spectra could be discriminated according to their pen varieties (category variables). Stepwise DA was performed by using the Mahalanobis distance method and probability-*F* as the criterion. Prior probabilities were equal for all groups (pen varieties) as the representativeness of population was unknown [12]. DA was used with jack-knife classification in the training set. Jack-knife classification is a type of cross-validation techniques that enables all of the available data to be utilized for training while still giving an unbiased estimate of the generalization capabilities of the resulting classifier [8].

Thirteen of 39 principal components that reduced from the selected IR region were selected by DA as predictor variables to form five linear discriminant functions (LDF). LDF 1 explains 62.5% of the total variance in the original data and was highly correlated (r=0.947) to category variable (pen varieties). LDF 2-5 accounted for less than 20% of the total variance. This means LDF 1 play the most important role in explaning differences among six different pen varieties.

Figure 2 shows the projections of the 72 spectra into the space of the first two discriminant functions, displaying 79.10% of the between-to-within group variation in the data. Based on Figure 2, three main clusters were detected. LDF 1 was found to be responsible for separation ‖19 20 21‖22 23‖24‖. In fact, each of the three clusters was represented by three different brands. Varieties 19-21; 22-23 and 24 belonging to the brands of G-soft, MGM and Pilot, respectively.

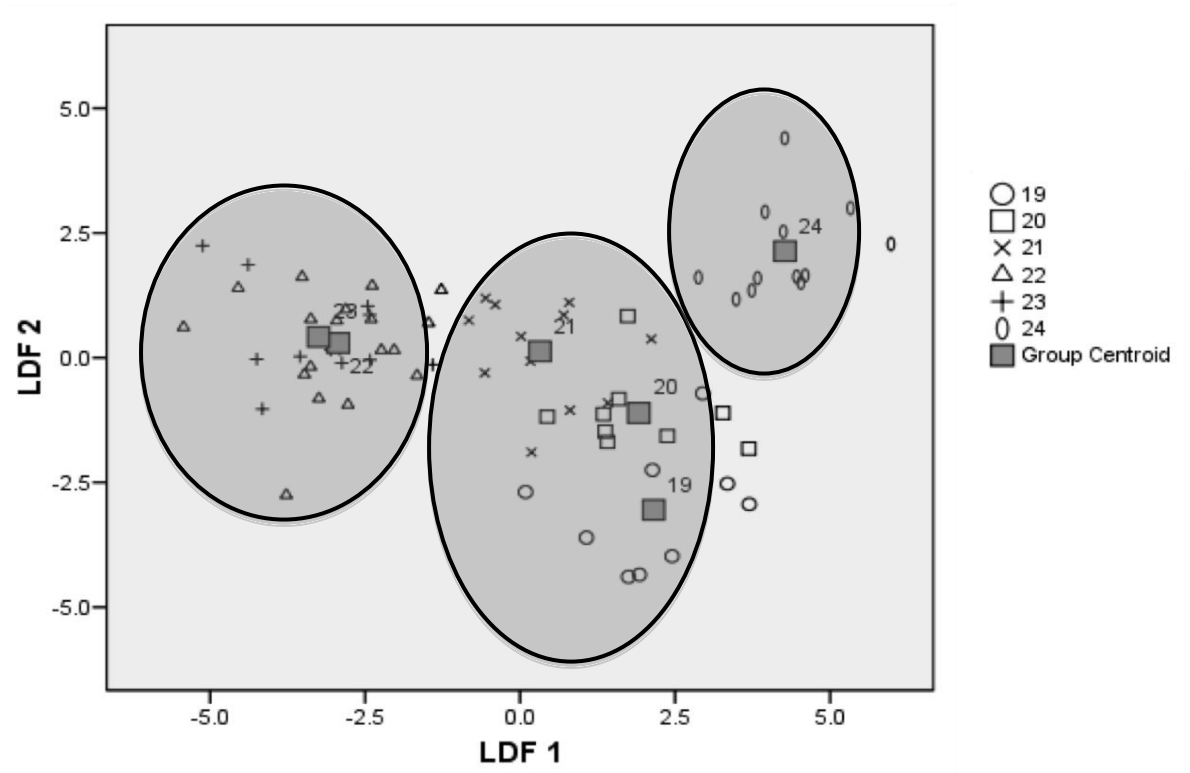

Figure 2: Scores plot of first two linear discriminant functions showing the separation of group centroids for six black ballpoint pen varieties.

Table 2 showed jack-knife classification results for the six groups (pen varieties) discriminant analysis. Out of the 72 spectra, 77.80% of IR spectral are classified correctly. The classification results also show overlapping groups. All the misidentification occured between varieties of a single brand, except for variety of 24. As such, if the DA was conducted to differentiate 72 spectra based on brands, higher correct identification rate could be expected.

Table 2: Jack-knife classification results for each variety.

Variety	Predicted group membership (%)						Overall hit rate (%)
	19	20	21	22	23	24	
19	78	11	0	0	0	11	77.80
20	0	100	0	0	0	0	
21	8	17	75	0	0	0	
22	0	0	0	72	28	0	
23	0	0	0	42	58	0	
24	0	8	0	0	0	92	

Differentiation Analyses

Pair-wise comparison was conducted to determine whether all six different pen varieties might be discriminated through the 39 predictor variables. With six different pen varieties, 15 ((1/2)K(K-1)) possible pairs have been created. One-way ANOVA was applied to find out whether the means of each predictor variable differed between pen varieties, to a 5 % significance level. Subsequently, appropriate post-hoc test was also conducted to determine the pairs of pen that differed significantly at particular variables. With the utilisation of the 95% confidence interval, there was a 5% probability of committing a type I error (false exclusion). When the confidence interval was increased to 99%, only a 1% chance (or less) of committing a type I error was allowed, however at the same time the probability of committing a type II error (false inclusion) increased. With respect to forensic casework, type II errors (indicating that two samples originated from the same source when they in actual fact do not) should be reduced or eliminated, if possible. In other words, although both types of errors pose problems for the forensic examiner, arguably from a forensic point of view data that presents a higher percentage of type II errors is preferred over the contrary [13].

Figure 3 shows the summary of results for 15 pen pairs. A discrimination power of 86.67% (13/15 X 100% = 86.67%) was achieved. Out of 15 pairs, only two pairs (20:21 and 22:23) were statistically indistinguishable suggesting that they share very similar (statistically the same) organic profile and were likely to have from the same source of origin. In fact, 20 and 21 as well as 22 and 23 were from brands of G-soft and MGM, respectively.

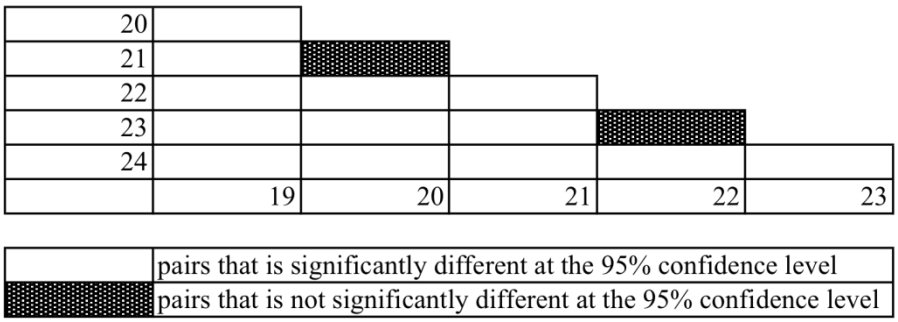

Figure 3. Univariate pair-wise group comparison of six black ballpoint pen varieties.

Conclusion

In conclusion, all six different pen varieties could be grouped into three clusters according to their brands via DA. Discrimination power of the proposed methods was 86.67%.

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Forensic Drug Profiling of Erimin-5 Using TLC and GC-MS

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ABSTRACT: Sixty-four groups of Erimin-5 tablets from 23 sources were profiled based on their dye and active ingredients. Dye of the tablets was extracted using 5% acetic acid and subjected to TLC separation using isopropanol/ammonia (4:1) as the solvent system while the active ingredients were analysed using GCMS and diluents were analysed using FTIR. All tablets were of peach-like or green in colour. The dye components were identified as tartrazine, sunset yellow, erythrosine, ponceau 4R and brilliant blue. The active ingredients were identified as nimetazepam and diazepam with glucose, sorbitol, mannitol and lactose as diluents. The combination of dye information and chemical contents allowed a quick classification of these 23 sources into six different profiles. This drug profiling method can provide useful information for narcotic enforcement and intelligence purposes. Forensic drug laboratories receiving tablet-form Erimin-5 should perform forensic profiling a profile database of the drug.

Keywords: Erimin-5, nimetazepam, dye, active ingredients, forensic chemistry

Introduction

Erimin-5 a brand name of nimetazepam initially used for the treatment of short-term severe insomnia in patients. Due to worldwide abuse, nimetazepam has been listed as a controlled substance in many countries [1]. In Malaysia, both nimetazepam and flunitrazepam, the two benzodiazepines are listed as control substances under the Dangerous Drugs Act 1952. While the latter is seldom encountered, the former tablets, either in the form of Erimin-5 or its counterfeits, were frequently submitted to the forensic laboratory for analysis [2].

Erimin-5 pills were frequently reported in illicit markets in Malaysia in the mid 1980s [3]. Its wide availability, relatively low price on the local black markets and its long activity has made it one of the most widely abused sedatives today. Heroin addicts use it as a substitute and it is also increasingly used as sedative by methamphetamine abusers after binging [4]. Illicit manufacturing and/or illegal smuggling of Erimin-5 is alarming. An estimated RM9.7 million worth of Erimin-5 pills were seized by the Royal Malaysia Police [5]. During the first eight months of 2012 alone, Malaysia police have seized a total of 4,115, 694 pills worth an estimated RM80 million [5].

Drug trafficker distributed the pills through their illicit marketing network. Therefore, profiling of these tablets can provide useful information [6] to help establish links among seizures [7] or to source of origins. Dye used in the tablets could provide useful information for forensic comparison between pills or between pills and dyes found in clandestine laboratories [8] or to a particular syndicate operation while active ingredients of the tablets help establish the chemical contents and legal status of a seizure. Therefore, this study aims to determine the dye components and active ingredients of Erimin-5 tablets obtained from 23 sources using routine Thin Layer Chromatography (TLC), GCMS (Gas Chromatography Mass Spectroscopy) and FTIR (Fourier Transform Infrared) analyses. Establishing a profile of these tablets may facilitate the law enforcement operations.

Materials and methods

All chemicals used were of HPLC grade. Acetic acid (5%), ammonia solution (3N) and iso-proponal:ammnia (4:1) were freshly prepared. The 64 groups of Erimin-5 tablets were obtained from seized samples from 2011-2012. Food colour standards were obtained from The Department of Chemistry, Malaysia.

Dye extraction and identification

Erimin-5 pills tablets were powdered using the mortar and pestle, acidified with 5% acetic acid in a beaker. Two knotted pieces of wool were submerged in the mixture which was warmed on a boiling water bath until the dye was transferred to the wool. The wool was then removed and washed thoroughly with water to remove other extraneous materials. The dye on the wool was then re-extracted by warming the wool with 5 mL of acetone and 3N ammonia mixture (1:1) on a water bath for approximately 5 min. The wool was then discarded and the dye extract was

allowed to evaporate until dry before being reconstituted in methanol. Dye standards were dissolved in methanol prior to TLC.

TLC separation was performed on 200 mm x 200 mm plates coated with 0.10 mm layer of silica gel. Dye extracts and standards were spotted on the plate using capillary tubes and developed in freshly prepared solvent system of isopropanol/ammonia (4:1) until the solvent front approached about 1cm away from the top of the TLC plate. The plate was marked and left to dry. Rf value of each band was then calculated.

Active ingredient and diluents identification

Erimin-5 tablets from each source were pounded with a mortar and pestle into fine powder. It is subsequently suspended into methanol and mixed well. The mixture was filtered and the filtrate was analysed using GCMS (Agilent GC-MS 6890N) equipped with an Agilent HP-5 capillary column (30 m length, 250 µm i.d., 0.25 µm film thickness) with a flow rate of 1.2 mL/min with helium as the carrier gas. The temperature of the injector was set at 260ºC. The initial oven temperature was set at 250ºC for one minute and then raised to 300ºC at a rate of 5ºC/min and held for two minutes.

For infrared analysis, the fine powder samples were analysed. Infrared spectra were obtained with a Nicolet Magna-IR Spectrometer 550. The resolution was set at 4.000 cm^{-1}, with 32 scans between 400 cm^{-1} and 4000 cm^{-1}.

Results and discussion

TLC Analysis

All 64 groups of Erimin 5 tablets were visually examined and grouped based on their colour. 53 groups were of peach-like colour and 11 groups were green. Tartrazine, Sunset Yellow, Erythrosine, Ponceau 4R and Red 2G were the standard dyes analysed together with the dye extract from peach coloured tablets were. Standard dyes used for comparing the dye extracts from green coloured tablets were Fast Green, Brilliant Blue, Green S, and Tartrazine.

Among the 53 samples of peach-like coloured samples, 18 contained a combination of three dyes, namely Erythrosine, Tartrazine and Ponceau 4R to produce the hue when the resulting bands were compared to the retention factor (R_f) of dye standards. The other 35 samples produced only one band suggesting the colour from the tablets comes from Sunset yellow. For the 11 green samples, two bands were observed which corresponded to those observed in the standard dyes of Brilliant Blue and Tartrazine suggesting that the colours from all of these tablets come from mixture of the two dyes.

Examinations on the dye composition of the 64 sample-groups from 23 sources showed that all samples from common sources had the same combination of dyes. This indicates that the respective tablets could be from the same origin hence can provide useful information to link the drug syndicates and delineate a drug syndicate's illicit market coverage.

The TLC analysis was shown to be rapid and cheap and enabled simultaneous assay of several dozen of tablets with satisfactory results [9]. The wool extracting technique was effective in extracting colouring substances from the tablets consisting of various substances.

GC-MS Analysis

The GC parameter employed gave the peak of diazepam at 5.546 min (Figure 1) and nimetazepam at 6.017 min (Figure 2), with glucose at 2.455 min and mannitol/sorbitol eluted at 2.714 min. About 82.8% (53/64) sample groups contained nimetazepam as the active ingredient. The remaining 17.2% (11/64) groups contained diazepam as the active ingredient.

Note that commercial Erimin-5 tablets should contain nimetazepam as the active ingredient. From GC-MS analysis, it was observed that the fake "Erimin-5" tablets from 11 samples of three different sources contained only diazepam as the active ingredient. This information is important to law enforcement personnel because only nimetazepam and flunitrazepam are the two benzodiazepines listed under the Dangerous Drug Act 1952.

Mannitol and sorbitol are isomers and the only difference is the hydroxyl group on carbon 2. So, this difference could not be detected using the GCMS without using derivation agent, so it yields the similar peaks for both compounds.

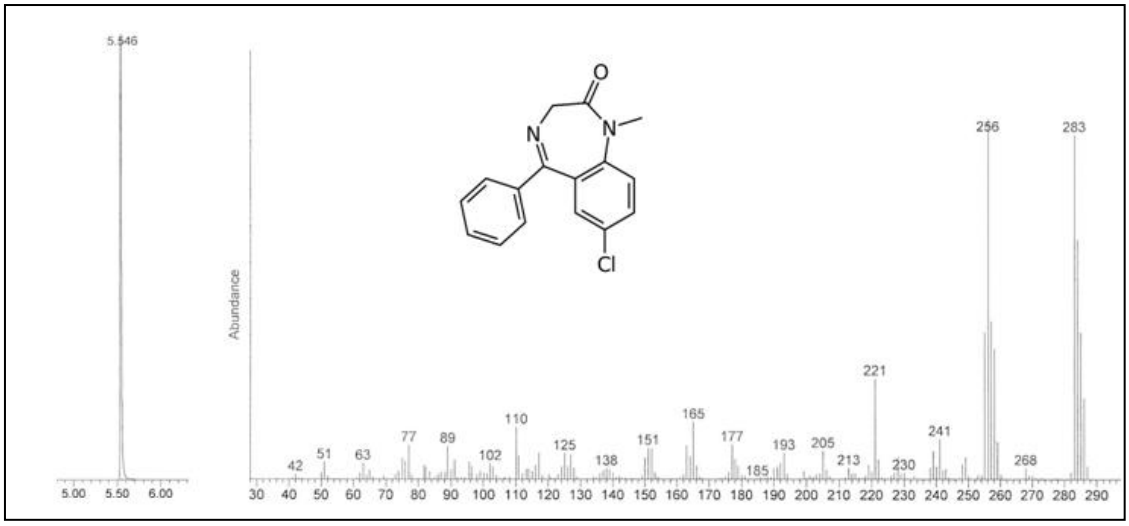

Figure 1: Gas chromatogram showing diazepam standard eluted at 5.546 min (left) and its mass spectrum (right)

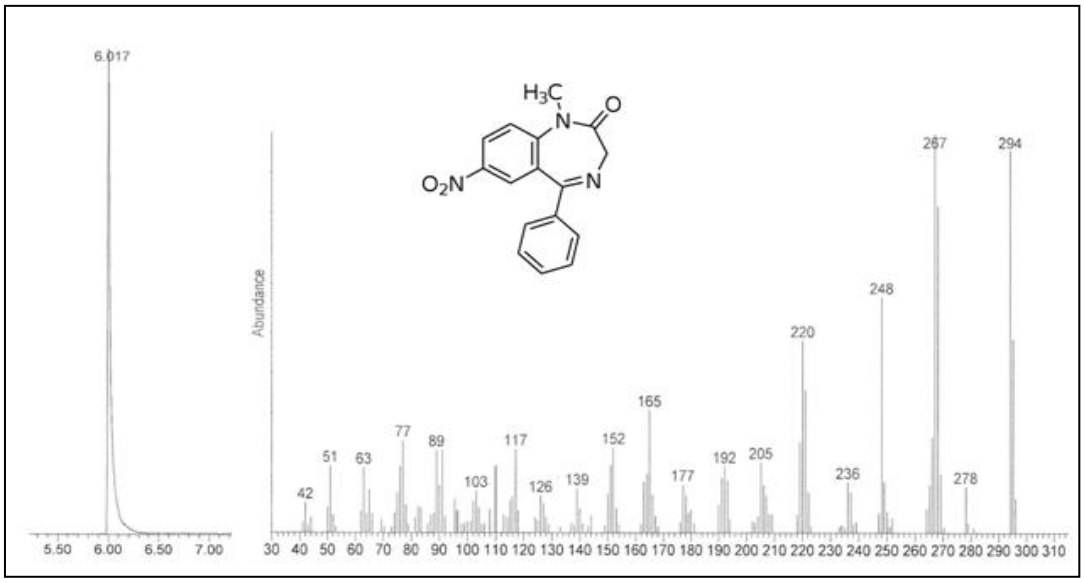

Figure 2: Gas chromatogram showing nimetazepam standard eluted at 6.017 min (left) and its mass spectrum (right)

FTIR Analysis

The FTIR analysis concluded three types of diluents in the samples that were analysed. FTIR technique has the capability to differentiate between the isomers, mannitol and sorbitol. Lactose, mannitol and glucose are the diluents identified in the samples through this technique. About 45.3% (29/64) samples contained lactose as the diluent. Seven (11.0%) samples contained glucose as the diluent and approximately 43.7% (28/64) samples contained mannitol as the diluent.

Erimin-5 profiling

Further profiling strategy were taken by combining the information of TLC, GC-MS and FTIR analyses as shown in Figure 3. It shows that the results from the three techniques enable the 64 samples for further classification into six profiles/clusters.

Tablets from six sources contain the active ingredient nimetazepam and lactose as diluent with a combination of Erythrosine, Tartrazine and Ponceau 4R to provide the hue. Tablets from nine sources contained nimetazepam, lactose and Sunset Yellow as the dye. Nimetazepam and mannitol as ingredients with Sunset Yellow as the colouring agent could be found from two sources. Only one source has sample

which contained diazepam and mannitol as ingredient and Sunset Yellow as the dye.

Tablets from three sources contained nimetazepam and mannitol with a combination of Tartrazine and Brilliant Blue to give the green colour Erimin 5. Tablets from two sources contained diazepam as active ingredient, glucose as diluent and mixture of Tartrazine and Brilliant Blue as the colouring agent.

Obviously, this simple profiling strategy could aid law enforcement operations who seized samples at different locations to link the source to its syndicate operation or source of illegal productions. Further profiling could also be made if more physical or chemical information could be generated.

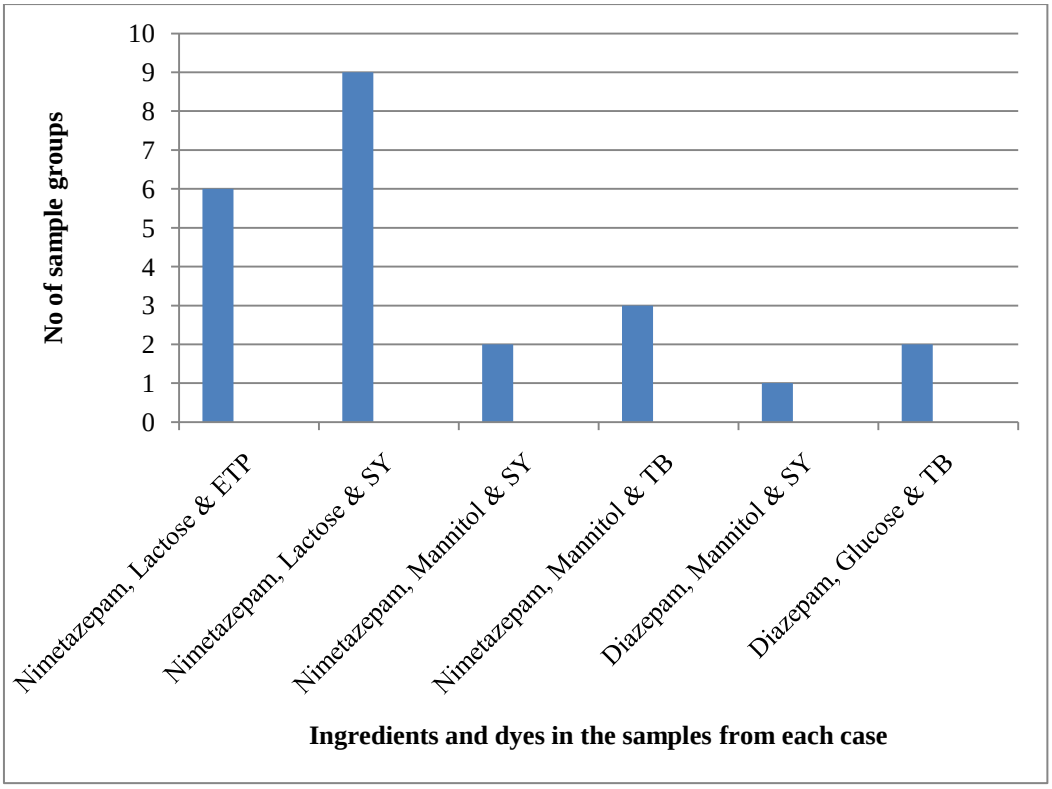

Figure 3: Bar chart shows the distribution of active ingredient, diluent and dyes according to number of sample groups (ETP = erythrosine, tartrazine and ponceau 4R, SY = sunset yellow, and TB = tartrazine and brilliant blue)

Conclusion

A framework of the Erimin-5 tablets profiling based on dye identification by TLC with active ingredient identification using GCMS and diluents identification using FTIR was developed. All the three analyses gave useful results, which were then used to compare the tablets from different sources. This rapid and cost effective drug profiling method can be easily adapted for forensic narcotic laboratories that normally receive Erimin-5 in tablet forms and could be used as routine laboratory procedure to build a drug profile database for investigative and intelligence purposes.

Acknowledgement

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Simultaneous Determination of Inorganic Ions in Post-Blast Residues using Capillary Electrophoresis

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ABSTRACT: Capillary electrophoresis method for simultaneous determination of inorganic anions and cations was developed using 2,6-pyridinedicarboxylic acid (PDC) as a background electrolyte (BGE) with indirect UV detection. In order to detect both anions and cations simultaneously, the electromagnetic flow was reversed by the addition of cetryltrimethylammonium hydroxide (CTAH). The parameters which influenced the separation of inorganic ions such as electrolyte pH, concentration of PDC, applied voltage and temperature were investigated. Four anions (Cl^-, NO_3^-, SO_4^{2-}, SCN^-) and three cations (Ca^{2+}, Fe^{2+}, Fe^{3+}) were successfully determined simultaneously in less than 7 min under the optimized conditions (25mM PDC, 0.5mM CTAH, pH 4.7). The method was applied to the analysis of post-blast explosive residues of black powder and ammonium nitrate-fuel oil (ANFO). The analytical performances of the method are discussed in terms of analysis time, repeatability, reproducibility, linearity of response and detection limits.

Keywords: Anions, cations, post-blast residue, capillary electrophoresis

Introduction

Post-blast residues, particularly inorganic residues refer to the debris and inorganic residue left at a crime scene from the detonation of explosive. Homemade explosive device used by terrorists are normally based on inorganic salts and/or peroxides since these ingredients are readily available, low cost and can be purchased legally [1]. The major components in explosives are oxidizers such as nitrate, chlorate, or perchlorate; and fuel such as charcoal, sugar, or metal oxides. After detonation, explosive leave residues that contain a variety of components such as ionic species which are produced from the explosive reaction, with un-reacted components of the original explosive mixture [2].

Determination of the level of inorganic anions and cations present in residue demands the identification of the original explosive [1]. Most of the chemicals species present in post-blast residues of low explosives are anions and cations. Examples of inorganic residue in most of the explosives are nitrite, nitrate, sulfate, chlorate, carbonate, perchlorate, ammonium, potassium and sodium [3-5]. The pattern of ionic components of post-blast residue often reflects the original composition, type, source and manufacturer of the explosive [6, 7].

Ion Chromatography (IC) has been the method of choice for the analysis of anions in post-blast residues [8]. Capillary electrophoresis (CE) is an attractive alternative for post-blast residue analysis because of its versatility. It can be applied to detect inorganic ions, organic molecules and large biomolecules using the same instrument and same capillary in most cases, given that the composition of running buffer is changed. This method is also environmental-friendly since the resultant waste contains only small amount of organic modifier and the buffer can be discarded without causing any danger to the environment [9]. CE can also yield a high speed and high resolution separation on small amount of sample [10]. Its availability for different modes of separation and its possibility in interfacing with different detection system makes CE widely applicable in various fields [9].

Recently, several CE methods for simultaneous analysis of cations and anions have been reported [5, 11]. Padaraukas *et al.* [11] proposed a unique technique based on electromigration sample introduction from both end of capillary using indirect UV detection with two background electrolyte (BGE). After applying voltage, anions and cations migrated in opposite direction from different capillary end and ions were detected at the detector which placed on the middle of the capillary. They successfully separated four anions and five cations in less than 5 minutes.

Hopper and coworkers [5] described the separation of inorganic ion using single injection with indirect UV detection. Single BGE was used with addition of

anion and cation chromophore, cation selectivity modifier and organic modifier. Eight anions and five cations were successfully separated with a total run of 7 minutes.

In this study, a new CE method for simultaneous analysis of anions and cations by using 2,6-pyridinedicarboxylic acid (2,6-PDC) as BGE was developed. The method was optimized and then applied for the simultaneous determination of inorganic ions in post-blast residues of black powder and ammonium nitrate-fuel oil (ANFO).

Experimental

Chemicals

Inorganic anion and cation standards were prepared from their salt. The chemicals used were of analytical or reagent grade. It included calcium chloride from QRec (Malaysia), ferrous (II) sulfate, ferric (III) nitrate, and potassium thiocyanate from GCE laboratory (USA). For electrolyte preparation, the chemicals used were 2,6-pyridine dicarboxylic acid from Fluka Chemica (Switzerland) and cetyltrimethylammonium hydroxide solution (25% in methanol) from Sigma-Aldrich (USA). For pH adjustment of buffer, sodium hydroxide solution was prepared from sodium hydroxide pellets from QRec (Malaysia). All electrolytes and standards were prepared using 18.2 MΩ double distilled deionised water (DDDW) from Milipore ultrapure water system (Merck, Darmstadt, Germany).

Instrumentation

CE instrument used in this study was an Agilent G1600 Capillary Electrophoresis System with indirect UV detection. For simultaneously separation of both anions and cations, the capillary used was a fused silica capillary with dimensions of 52 cm length x 50 µm I.D. CE ChemStation software was used for system control, data collection and data analysis.

Electrophoretic Procedure

The electrolyte was prepared containing 0.5 mM cetyltrimethylammonium hydroxide (CTAH) to reverse the direction of electroosmotic flow (EOF) [12] and BGE at a concentration of 25 mM. The pH was adjusted to 4.7 with 1 M NaOH. Prior to use, the capillary was pretreated with the following procedures: deionised water for 10 minutes, 0.1 M NaOH for 10 minutes and deionised water for 10 minutes. The capillary was pre-conditioned with 2,6-PDC buffer for 4 minutes for each run. Samples were injected in the hydrodynamic mode for 6 seconds. The capillary temperature was held at 15ºC and the applied voltage was at -20 kV. Detection was carried out using UV diode array detector and the signal wavelength was set at 350 nm with reference at 230 nm.

Preparation of Standards

All standards (Cl^-, NO_3^-, SO_4^{2-}, SCN^-, Ca^{2+}, Fe^{2+}, Fe^{3+}) were initially prepared at a concentration of 1000 ppm and diluted to a working concentration of 100 ppm. The solutions were sonicated for 5 minutes and filtered through 0.45 µm membrane filter disc. A series of standard mixture solution ranging from 20 ppm to 100 ppm were produced which were used for the preparation of calibration curve. The LODs of each ion were evaluated by diluting 20 ppm standard mixture with DDDW and analyzed using the developed CE method. The lowest detectable concentration for each ion was based on signal-to-noise ratio of 2:1.

Sampling

Samples of post-blast black powder residues were obtained from a previous sampling exercise [18] at Rompin, Pahang. Three fragments (BP-1, BP-2 and BP-3) were collected as post-blast residues of different explosive charge of 100 g, 150 g and 200 g, respectively.

For ANFO residues sample, a homemade bomb was made by police personnel at the sampling site using 30 g of ammonium nitrate mixed with diesel fuel oil that was filled into a plastic mineral bottle. A detonating cord containing PETN as a booster was attached to the plastic bottle. The homemade bomb was placed in the middle of a field and two different types of cloths (cotton and nylon) were placed 2.5 meters away from the bomb in a position 180º from each other. The cloths (23 cm x 32 cm) were tied to a metal plate fixed onto a small iron rod. After detonation, each cloth was collected and packed in snap-seal plastic bag.

Sample Preparation

i. Sample Preparation of Black Powder Residue

For swabbing of black powder residue from bomb fragments, the cotton balls were pre-cleaned before use. The cotton balls were first cleaned by soaking them in DDDW for 15 minutes. The cotton balls were taken out and excess water squeezed out. The cotton balls were left to air dry for about 20 minutes. The pre-cleaned cotton balls were picked up using forceps and moistened with DDDW. The moist cotton ball was swabbed gently onto the surface of post-blast fragments to collect the residue.

Cotton swab containing the residue was placed in a 100 mL sample bottle and filled with 20 mL of DDDW. The sample bottle was sonicated for 10 minutes to extract the inorganic ions. After sonication, the cotton swab was removed from the sample bottle and the resultant solution was filtered through a cleaned 0.45 µm membrane filter disc. The extract was diluted to 100 mL, followed by degassing for 5 minutes. The extract solution was placed in a

sample bottle and kept in a refrigerator prior to analysis.

ii. Sample Preparation of ANFO Residue

For ANFO residue deposited on cloth, the cloth was cut into smaller pieces and placed in a 50 mL sample bottle. Twenty mL of DDDW was added into the sample bottle followed by sonication for 10 minutes in order to extract the inorganic ions. The cloth pieces were removed from the sample bottle and the resultant solution was filtered through 0.45 µm membrane filter disc and diluted to a total volume of 25 mL. The extract solutions were degassed for another 10 minutes and kept in the refrigerator prior to analysis.

Analysis of ANFO Control Sample

ANFO control sample used in the study was provided by the Royal Malaysia Police. A 0.01 g of ANFO control sample was dissolved in 10 mL volumetric flask using DDDW. The mixture was sonicated and filtered through a 0.45 µm membrane filter disc. A 100x dilution was carried out by dissolving 1 mL of ANFO solution in 100 mL volumetric flask using DDDW. The diluted standard solution was kept under refrigeration prior to analysis.

Results and Discussion

Choice of BGE

For simultaneously separation of anions and cations, an indirect UV detection was utilized. This detection mode required that the electrolyte contained both cationic and anionic visualization agents. In this study, 2,6-PDC acid was chosen as BGE due to its ability to form negatively charged complexes to permit indirect UV detection. 2,6-PDC is also known to have high sensitivity, high stability and UV responsive for metal cations [13, 14]. Formation of anionic complexes was also found to be highly dependent upon electrolyte pH and concentration of 2,6-PDC acid [15].

Once 2,6-PDC acid reacted with cations to form anionic complexes, they migrated toward positive electrode and allow for indirect detection. Indirect UV detection was employed in this study to visualize anions which have little and no chromophore. Through indirect detection, anions were recorded as positive peak while cations were recorded as negative peak. For reversing the direction of electroosmotic flow (EOF) from cathode to anode, a cationic surfactant that comprised of cetyltrimethylammonium hydroxide (CTAH) was added to the BGE.

A simultaneous separation of both anions (Cl^-, NO_3^-, SO_4^{2-} and SCN^-) and cations (Ca^{2+}, Fe^{2+} and Fe^{3+}) were successfully achieved using 2,6-PDC. Four anions and three cations were eluted within 7 minutes (Figure 1). Although the cations were detected as negative peaks, the PDC electrolyte provided a satisfactory separation of all seven components. All peaks were baseline separated except for Cl^- and NO_3^- peaks that were not well resolved. Another peak that eluted between Fe^{2+} and Ca^{2+} peaks indicated the presence of a solvent peak by comparison with the electropherogram of the blank extract.

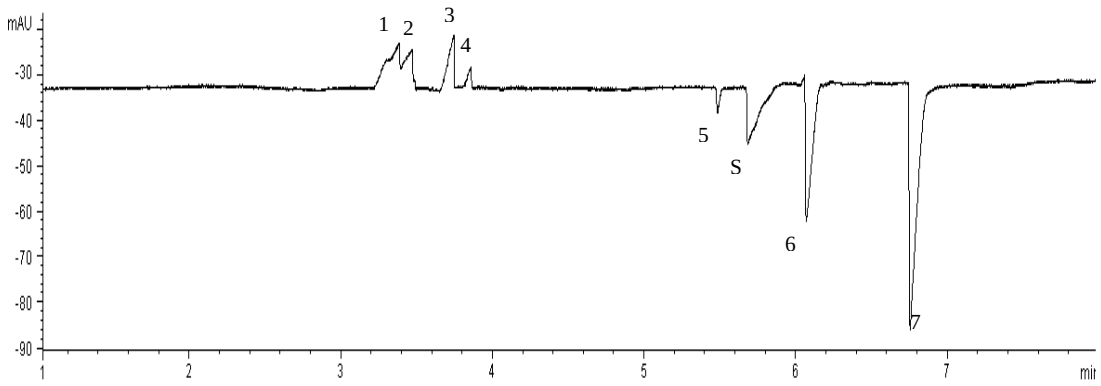

Figure 1: Electropherogram of inorganic anions and cations. Concentration of analytes at 100 ppm. Conditions: fused silica capillary (52 cm x 50 µm I.D.); potential: -20kV; capillary temperature: 15ºC; electrolyte: 25 mM 2,6-PDC and 0.5 mM CTAH (in 25% methanol); buffer pH 4.7; injection: 6.0 s x 50.0 mbar; wavelength: 350 nm, 20 ref. 230 nm, 10 ref. Peak identification: (1) Cl^-; (2) NO_3^-; (3) SO_4^{2-}; (4) SCN^-; (5) Fe^{2+}; (6) Ca^{2+}; (7) Fe^{3+}, (S) solvent.

Optimization of CE Separation

i. Effect of BGE pH

Buffer pH would significantly have an effect on EOF because of an increase in the dissociation of silanol and silanoate group on the inner capillary wall [16]. As shown in Figure 2, migration time for most of the analytes generally increased with an increase in pH. However, a change of separation selectivity was obtained for Ca^{2+} and Fe^{3+} ions at pH 3.7 and pH 4.7. Fe^{3+} migrated earlier than Ca^{2+} at pH 3.7, but migrated later than Ca^{2+} at pH 4.7. This is because 2,6-PDC is an ionisable compound in which its ligand concentration depends on pH. Increasing pH will favor the protonated ligand concentration and also increases the degree of metal complexation. Besides that, pH changes will affect both separation selectivity and detection sensitivity [17].

An optimization of electrolyte pH was seen between pH 3.7 to 5.7 where the migration times of analytes were more stable. From Figure 3, it can be seen that Ca^{2+} could not be detected beyond pH 7.7 while, Fe^{2+} could not be detected at pH lower than 3.7. Therefore, pH 4.7 was selected as the optimized pH for subsequent experiment.

ii. Effect of PDC Concentration

Concentration of buffer also has an influence on EOF without altering the selectivity as pH. By increasing the buffer concentration, it will lower the EOF while lower buffer concentration will give shorter analysis time [18]. With a constant concentration of 0.5 mM CTAH in the electrolyte run, the concentration of 2,6-PDC was varied from 10 to 30 mM at pH 4.7 (Figure 3). Migration time of anions increased slightly with increasing BGE concentration while migration time of cations were found to increase significantly with increasing buffer concentration.

At a BGE concentration of 10 mM, the NO_3^- peak was difficult to observe and was not separated completely from Cl^- and SO_4^{2-} peaks while Fe^{2+} peak appeared closely to the solvent peak (Figure 3A). In addition, at 30 mM BGE concentration, the SO_4^{2-} and SCN^- peaks were not resolved and an increase in baseline noise was observed (Figure 3E). Under all BGE concentration employed, Cl^- and NO_3^- peaks were not separated completely. This could be due to insufficient length of the capillary used. However, resolved anionic peaks of Cl^- and NO_3^- were observed at 25 mM of 2,6-PDC. Hence 25 mM buffer concentration at pH 4.7 was selected as the optimum BGE condition.

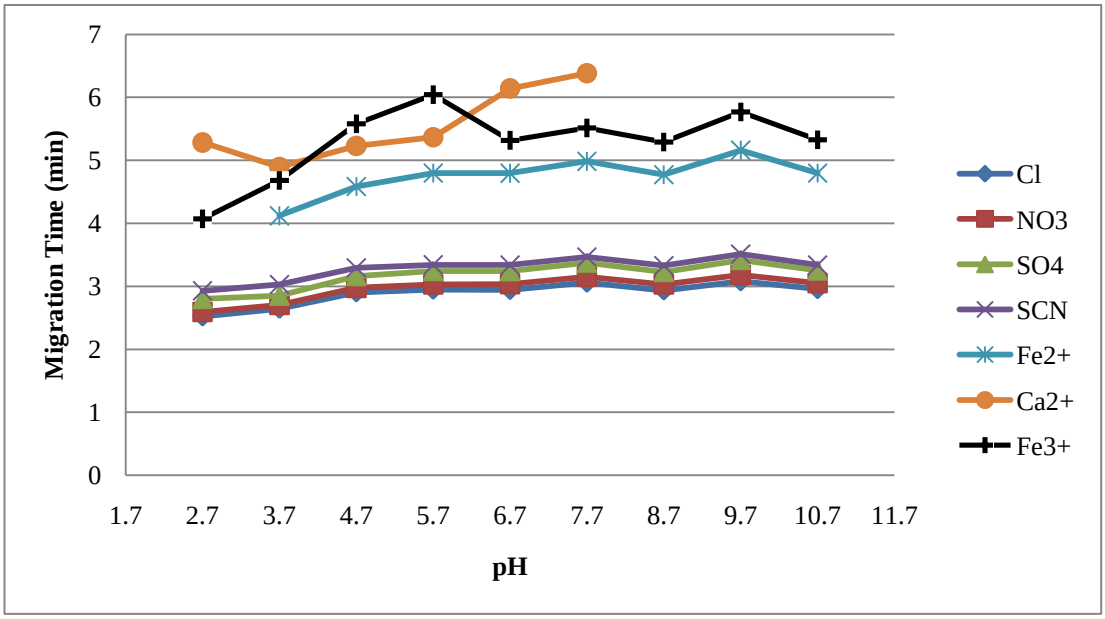

Figure 2: Effect of electrolyte pH on migration time

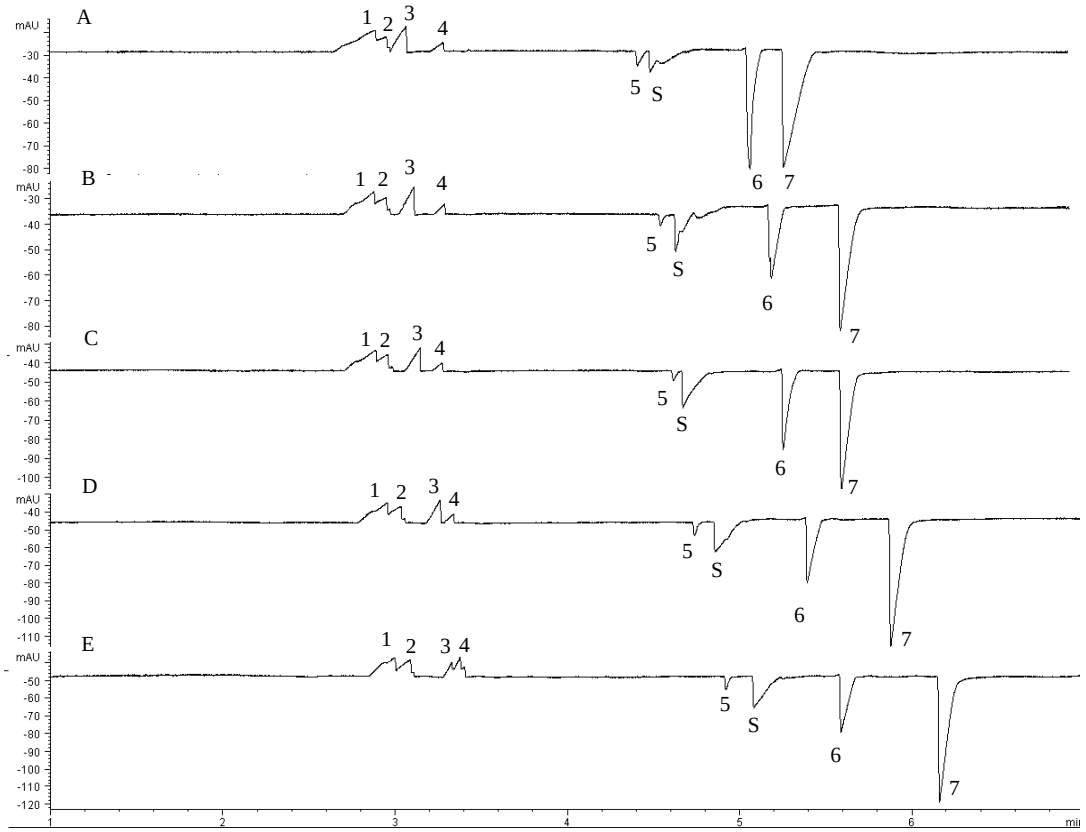

Figure 3: Effect of electrolyte concentration on peak separation. Concentrations of analytes at 100 ppm. Potential: -20kV; capillary temperature: 20ºC; electrolyte: 0.5 mM CTAH with 2,6-PDC. (A) 10 mM, (B) 15 mM, (C) 20 mM, (D) 25 mM, and (E) 30 mM, pH 4.7. Peak identification: (1) Cl^-; (2) NO_3^-; (3) SO_4^{2-}; (4) SCN^-; (5) Fe^{2+}; (6) Ca^{2+}; (7) Fe^{3+}, (S) solvent.

iii. Effect of Potential

The effect of potential was studied by the varying voltage from 15 kV to 25 kV as shown in Figure 4. Results indicated that by increasing the applied potential, the analysis time was typically reduced. In contrast, at lower negative potential, the migration time was increased. In this study, although separation with short migration time was observed at -25 kV, adequate separation was observed at -20 kV with less current produced. Therefore, a potential of -20 kV was selected as the optimal running potential.

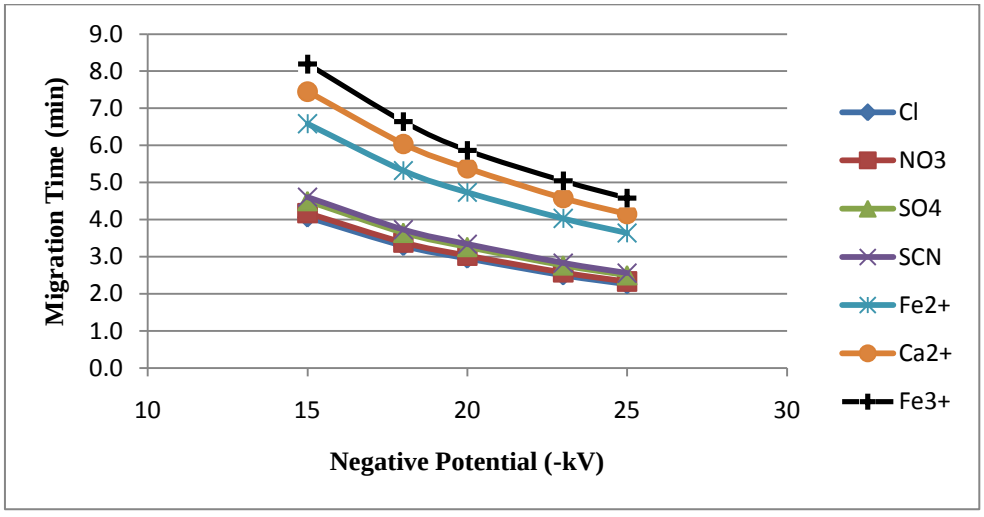

Figure 4: Effect of applied potential on migration time.

iv. Effect of Temperature

Figure 5 illustrates the migration times of target analytes in the temperature range from 15 to 30ºC. Migration time was found to decrease with increasing temperature. A noisier baseline was also observed at higher temperature. A rise in temperature causes an increase in EOF due to a decrease in the viscosity of the buffer. An increase of 1ºC will generally reduce the viscosity of water by 2.4% [16]. Therefore, temperature was also considered as important in separation since both analyte mobility and level of EOF are temperature related. In this study, baseline drifts were observed at temperatures approaching 30ºC. An optimum applied temperature for the best separation was therefore selected at 15ºC.

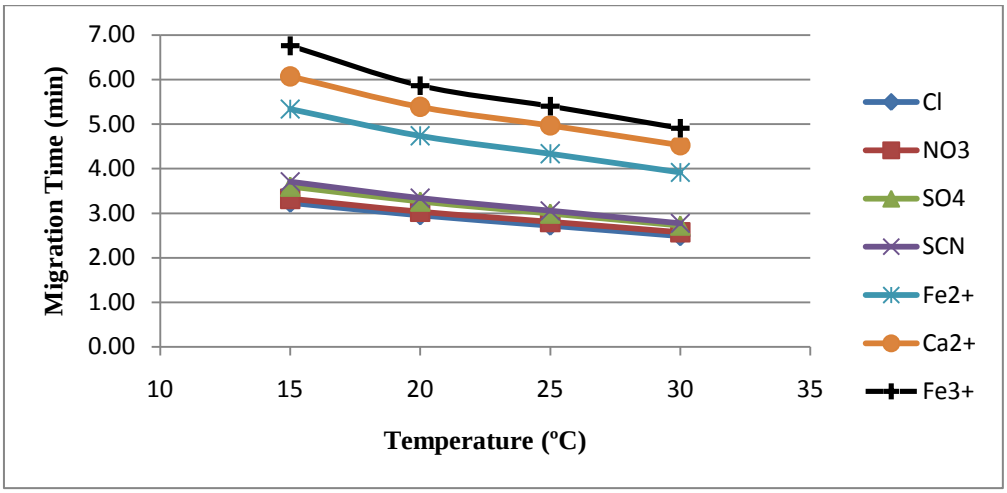

Figure 5: Effect of temperature on migration time.

Method Validation and Repeatability

A calibration plot of anions and cations was generated to determine the concentration of analyte ions in post-blast residues. Calibration curves were plotted for the respective inorganic ions within the concentration range of 20 to 100 ppm. The correlation coefficients (r^2) and detection limits (LODs) are listed in Table 1. The correlation coefficients (r^2) of each calibration curve were excellent (> 0.986) that proved good linearity of the present method. The detection limits for anions were in the range from 2 ppm to 15 ppm, while cations were in the range from 0.4 ppm to 3 ppm using a 50 mbar/s pressure injection at a signal to noise ratio of 2:1. Limits of detection from this study were compared with previous studies and it found that LODs in this study were much lower except for Ca^{2+}. The differences of LODs obtained from the present study and the earlier studies (Table 1) may be due to the different parameters employed in CE method.

Table 1: Correlation coefficients *and* detection limits of target analytes

Ion	**Correlation coefficient (r2)**	**LOD (ppm)**	
		This work	**Other work#**
Cl-	0.990	2.0	8.0a
NO3-	0.996	3.0	11.0a
SO42-	0.988	3.0	9.0a
SCN-	0.986	15.0	-
Ca2+	0.995	2.0	0.4b
Fe2+	0.987	0.4	0.6c
Fe3+	0.988	3.0	6.0d

#References: [a]Jimidar *et al.* [20]
[b]Chen and Naidu [17]
[c]Lutzenkirchen and Lovgren [21]
[d]Harrold *et al.* [22]

A method is considered precise when reproducible migration times and peak areas are obtained. Precision can be determined by performing repetitive analyses of the same sample within a system under the same condition and calculating the relative standard deviation. A standard ion mixture of 100 ppm was injected three times per day and also in six consecutive days respectively. The reproducibility of the method is tabulated in Table 2. The reproducibility of migration time was found to be better than 0.18% for within day variation. For day-to-day variation, the reproducibility of migration time was found to be better than 0.31%. In addition, the reproducibility of peak areas was found to be better than 2.97% for within-day and 3.60% for day-to-day variation (Table 2). The results show good reproducibilities obtained for both migration time and peak area as reflected by the % RSD for within day (n=3) and day-to-day (n=6).

Determination of other cations including alkaline metal was also studied; however, no peaks were detected. This is because there is no change in UV absorbance between cation-complexes and PDC electrolyte. These cations might form negative complexes with PDC but due to its lower charge, the negative complexes could not be observed [13, 15].

Table 2: Within day and day-to-day reproducibility of target analytes

Ion	% RSD Migration Time		% RSD Peak Area	
	Within day	Day-to-day	Within day	Day-to-day
Cl^-	0.031	0.236	2.206	1.431
NO_3^-	0.064	0.181	2.103	2.677
SO_4^{2-}	0.074	0.283	1.967	2.146
SCN^-	0.042	0.312	2.973	0.437
Ca^{2+}	0.085	0.208	0.826	2.439
Fe^{2+}	0.049	0.222	1.238	0.548
Fe^{3+}	0.181	0.181	2.623	3.598

Separation of ANFO Control Sample

In CE separation of ANFO control sample, only NO_3^- was successfully identified (Figure 6). Ammonium ion could not be detected by CE due to its lower charge that causes the negative complexes not to show any changes in UV absorbance [15]. Nitrate was present in large amount in the explosive mixture as indicated by the intense peak in the electropherogram. This peak indicated nitrate as an important component in ANFO explosive.

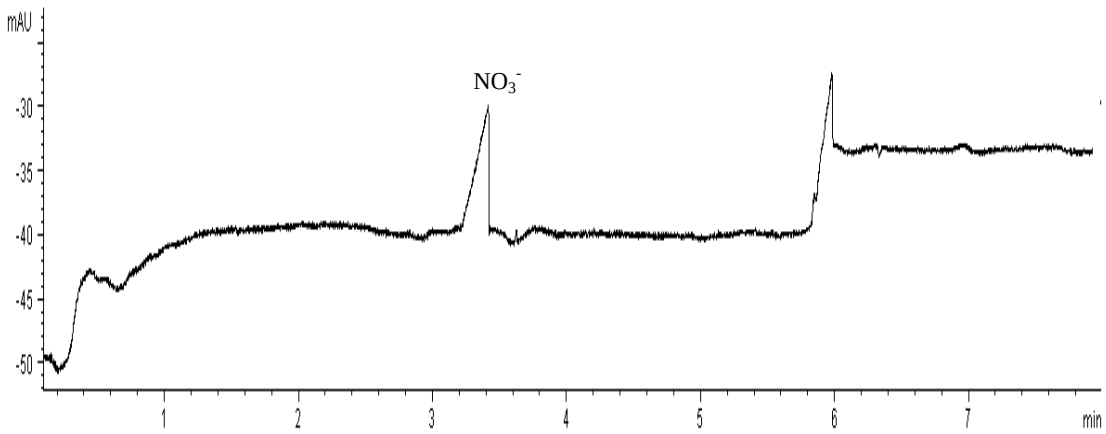

Figure 6: CE separation of ANFO control sample. CE conditions as in Figure 1.

Post-Blast Residue Analysis

The developed method was applied to the determination of anions and cations in post-blast residue of black powder and ANFO. Black powder residue from three post-blast samples labeled BP-1, BP-2, and BP-3 were analyzed. CE separation revealed four (Cl^-, NO_3^-, SO_4^{2-} and Ca^{2+}) ions in sample BP-1 and three ions (NO_3^-, SO_4^{2-} and Ca^{2+}) in sample BP-2 and BP-3 (Figure 7).

Black powder is known to consist of potassium nitrate (KNO_3), charcoal (C) and sulfur (S). Therefore the presence of nitrate ion (NO_3^-) and sulfate ion (SO_4^{2-}) are indicative of black powder as the explosive material used as follows.

$$10\ KNO_{3(s)} + 3\ S_{(S)} + 8\ C_{(S)} \rightarrow 2\ K_2CO_{3(S)} + 3\ K_2SO_{4(S)} + 6\ CO_{2(g)} + 5\ N_{2(g)}$$

The presence of calcium (Ca^{2+}) and chloride (Cl^-) can be explained through the principal ingredients of homemade explosives which consist of fuels and oxidizers. Fuels that are commonly used include sugar, sulfur, or metal such as calcium, aluminium, magnesium and zinc. These substances are mixed with inorganic oxidizer such as salt containing nitrate, chlorate or perchlorate. During explosion, these chemicals undergo chemical reaction to produce ionic species in post-blast residue [2]. According to McCord *et al.* [6], the presence of chloride is a result of the chemical reduction of chlorate from perchlorate oxidizer as follows.

$$ClO_4^- \rightarrow ClO_3^- \rightarrow Cl^-$$

Analysis of ANFO residue deposited on cloth (cotton and nylon) was also conducted using the developed method. A minor peak due to nitrate (NO_3^-) was identified in the cotton extract. For further confirmation of the small peak in cotton extract, NO_3^- standard of known concentration was spiked into cotton extract and CE analysis was carried out. The analysis showed enhancement in peak area that indicated the presence of NO_3^-in the cotton extract. Nitrate was not detected in nylon extract possibly due to low amounts of ANFO residue that adhered to nylon. Nylon is a man-made fiber which is smooth and renders the residue difficult to adhere onto the fiber. The presence of NO_3^- indicated the use of ANFO since the major ingredient in ANFO is ammonium nitrate.

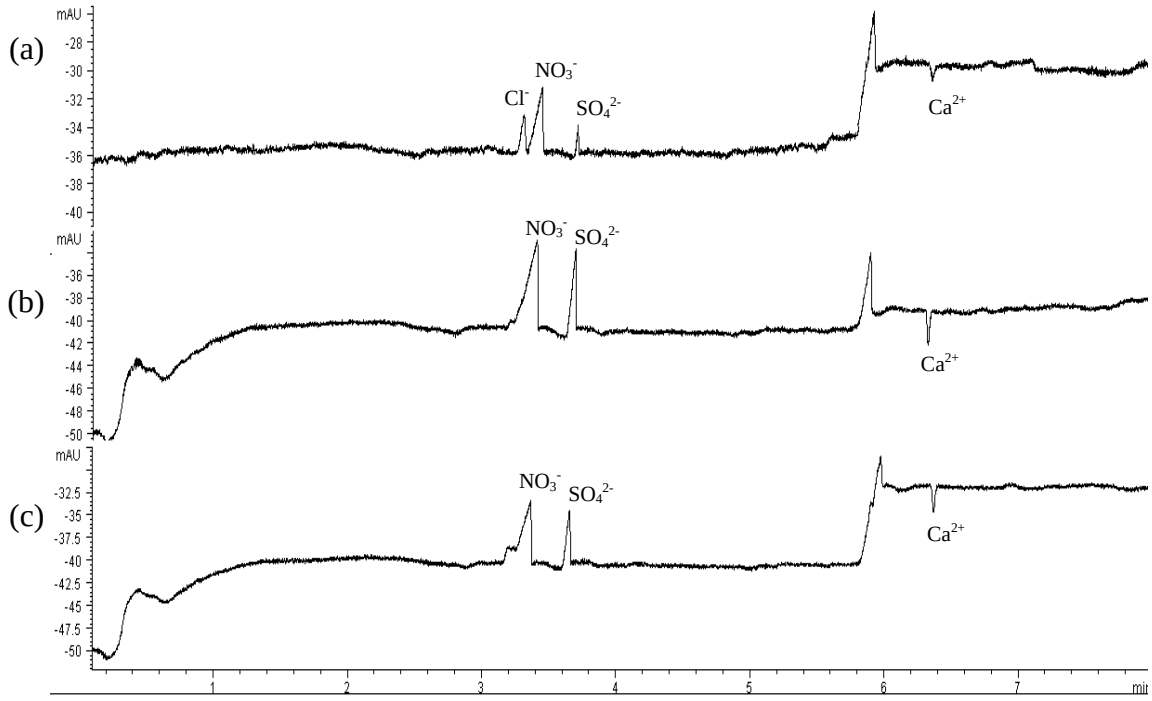

Figure 7: CE profile of black powder residue from three post-blast samples (a) BP-1, (b) BP-2, (c) BP-3. CE conditions as in Figure 1.

Conclusions

A reliable and rapid method for simultaneous determination of cations and anions using CE has been developed. This method offers the advantages of fast analysis, minimal sample consumption and capable of separating inorganic anions and cations simultaneously in a single run and with good linearity and reproducibility. The developed CE technique was applied for the detection of selected inorganic ions in several post-blast residues of explosive samples. Although CE could not detect several cations of forensic interest (Na^+, K^+ and NH_4^+), detection of three anions (Cl^-, NO_3^- and SO_4^{2-}) along with three cations (Fe^{2+}; Ca^{2+} and Fe^{3+}) indicated the use of black powder in the explosive. Meanwhile, detection of NO_3^- indicated the use of ANFO. However, the detection limits obtained (0.4-15 ppm) does not yet satisfy the requirements for real post-blast residue analysis. CE pre-concentration of analytes based on stacking or other techniques can be further developed to address this problem.

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Identification of Fuel Oil in Absorbent and Non-absorbent Surfaces in a Site of Ammonium Nitrate-Fuel Oil (ANFO) Blast

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ABSTRACT: The investigation of explosion covers incidents ranging from accidents in the home or workplace to major terrorist attacks. In the scene of an explosion, the forensic scientists try identify the kind of explosion occurred, the materials involved and assists the police investigation. Legitimate explosives include fireworks and blasting materials used in quarrying. The current trend involves misuse of certain legitimate chemicals, widely available to the general public, as precursors to homemade explosives. ANFO on quarry explosives remains one of the most commonly used products in quarry blasting. The explosive is misused by the perpetrators for criminal activities such as blast fishing, Automated Teller Machine [ATM] bombings and terrorism. Another form of blasting activities wherein the unskilled perpetrators prepared IEDs with improper weight ratio of AN and FO to trigger explosions which caused ineffective explosions and damages that are reflected in the crime scene. The objectives of this ANFO blasting project were to identify the presence of oil residues on absorbent and non-absorbent surfaces in sites of blasts; to study the extent of fuel oil travels from the blast crater after the blast; and to study different ANFO blastings using different AN and FO mixing proportions. The result of controlled ANFO surface blasting study showed the possibility of identifying the fuel oil in different surfaces placed in different known distances. The formation of a fuel oil flash pattern in the crater reflected the improper fuel and oil combination that formed a valuable finding in the ANFO blast scene investigation.

Keywords: Forensic science, ANFO blast, absorbent & non-absorbent surfaces, flash pattern.

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Introduction

Fire and explosions are both the result of combustion reactions, where a fuel and oxygen react, sometimes violently and instantaneously, to give off large amounts of energy [1]. Fuels commonly found in commercial explosives include diesel fuel, carbon, PETN, TNT, smokeless powder, monomethylammonium nitrate, and monoethanolammonium nitrate [2]. The most common oxidizer used for explosion is ammonium nitrate, sometimes sodium nitrate and calcium nitrate [2]. Explosives are solid, liquid or gaseous substances which, when suitably initiated, suffer rapid decomposition with liberation of heat and production of large volumes of gas at high pressure. [3]. Explosives are used for a wide variety of purposes but their main applications lie in mining and quarrying and in construction and demolition work [4]. Ammonium Nitrate-Fuel Oil (ANFO) remains one of the most commonly used products in quarry blasting. ANFO, a mixture containing 94% AN and 6% fuel oil, is probably the most commonly used explosive material in the world today [5].When it is used illegally and to cause harm it is generally known as a bomb. The current trend is the problem of the misuse of widely available chemicals such as ammonium nitrate as precursors to homemade explosives.

Knowledge of preparing explosives from ammonium nitrate (AN) and common availability of ingredients are used by offenders and terrorists [6]. ANFO is inexpensive and safe to handle. The availability of AN in the form of fertilizers makes it a readily obtainable ingredient for homemade explosives [7]. Home-made explosives, in turn, are the tool most preferred by terrorists and other criminals to perpetrate attacks. While the majority of homemade bombs are still simple in their construction and functioning, the percentage of the more complex bombs employed has risen [8]. The analysis and detection of explosives continues to be a global issue of prime importance.

The forensic science community is interested in examining the blasting scenes and analyzing post-blast explosive residues, chemicals and materials associated with bomb making [9]. AN and FO are neither explosive by itself, but, is a high power explosive when mixed in the proper weight ratio. ANFO mixtures are a favourite of car and truck bombers. It was the explosive used and identified in 1993 the world trade centre bombing[1], 1995 bombing of the Murrah federal building in Oklahoma city[1], 2005 bombing of rush-hour London buses and trains, 2010,2011 serial bombings in India and so on. The aim of this pilot ANFO blasting project, the first kind in Malaysia was to identify the presence of fuel oil residues on absorbent and non-absorbent surfaces in a site of ANFO blasts, and to study the extent of fuel oil travels from the crater after the blasts and also to study ANFO blastings using different AN and FO proportions.

Materials and methods

Place of blasting exercises

The blasting exercises were conducted with the technical assistance from Tenaga Kimia Sdn Bhd campus, Batu Arang, Selangor (Fig 1) upon obtaining permission from the CEO and police.

Fig1: Tenaga Kimia Sdn. Bhd. factory campus, Batu Arang, Selangor, Malaysia

Absorbent and nonabsorbent surfaces used to collect post blast fuel oil residue

Selected absorbent surfaces like kitchen towel pieces, A4 paper, soil, dry leaves, cotton, plywood and nonabsorbent surfaces like aluminum sheets, PVC sheets, glass plates, rubber sheets, plastic sheets, ceramic tiles were used to obtain oil residue after the blasts. These surfaces were kept side by side in circular form around the centre point of explosion. The blasting exercises were conducted by keeping the surfaces from the centre point to distances of 5m, 7m and 9m radius as shown in figure 2.

The circle was divided into four parts and each part consists of six different types of absorbent surfaces. Hence, there were 24 non-absorbent surfaces in one circle or distance. Blasting exercises were conducted by keeping the absorbent and non-absorbent surfaces in intact around the crater point from a distances of 5 m, 7 m and 9 m respectively.

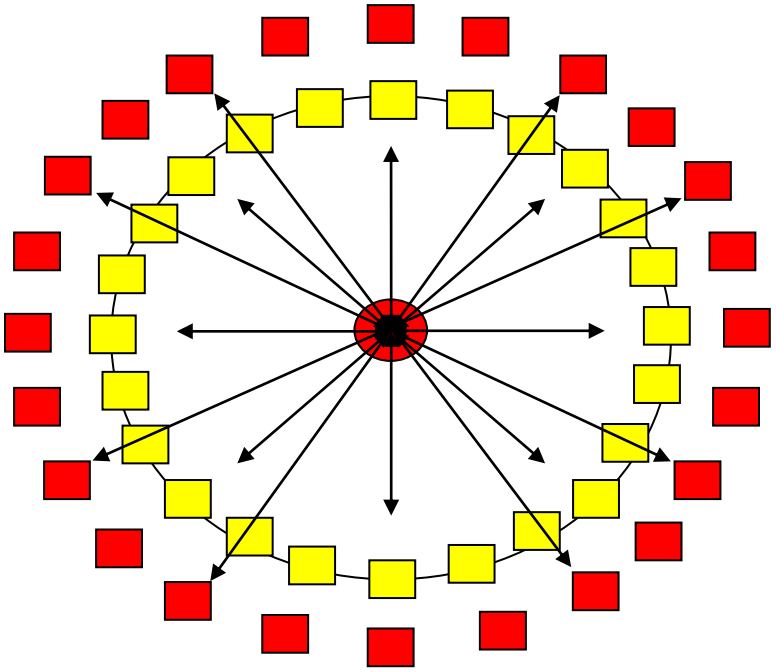

Fig 2: Arrangement of absorbent & non-absorbent surfaces around the blasting point

Chemicals used for blasting
Ammonium nitrate prills
Diesel oil
Emulsion explosive as booster [Emulux 150]
Electric detonator

Apparatus and other materials used
Measuring cylinder (10 mL)
Beaker (100mL)
Balance
Glass droppers
Measuring tape
Gloves
Rubber bands
Raffia ropes
Packing materials
Bricks to support

Preparation of ANFO
Since ANFO is a secondary high explosive, three-step explosive train was required to conduct the blasting exercises. In this study, three-step explosive train consists of electrical detonator, emulsion explosive and ANFO mixture. Instant electric detonators with No. 8 cap and Emulex® 150 (25x200 mm, 0.120 kg) as emulsion explosives were used for the blasting exercise as shown in figure 3. As per norms, ANFO comprises of explosive-based AN prills and commercial diesel fuel. As per the research requirement, ANFO mixtures were prepared in various proportions as shown in Table 1. Other devices used in this exercises were digital ohmmeter, blasting machine and connecting wires.

Table 1: Varying proportions of AN and FO

No.	Ratio of ANFO (by weight)	AN (kg)	FO (ml) (Diesel fuel)
1	94:6 (standard)	0.5	38.35
2	90:10	0.5	66.78
3	80:20	0.5	150.24
4	60:40	0.5	400.60
5	50:50	0.5	600.96

** Density of FO (Diesel) = 0.832 kg/L

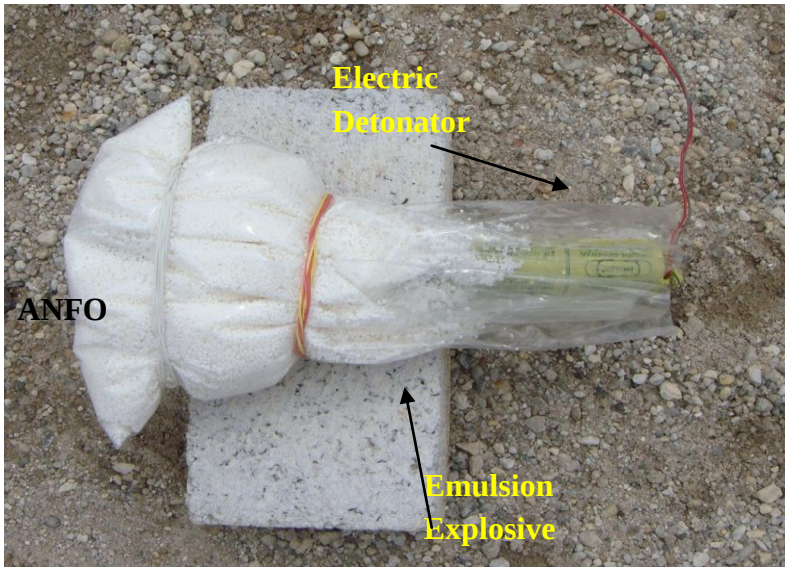

Fig 3: Three-step explosive train used in the blasting exercises.

Blasting procedure and sample collection

The plastic bag containing ANFO mixture (mixing ratio of 94:6 by weight for standard) was connected to detonator through booster was placed on a designated centre point in the blasting site surrounded by absorbent and non-absorbent surfaces placed at known distances as shown in Figure 2 and Table 2.

The leg wires of the instant electric detonator were connected to the blasting machine through the firing cable forming an electric circuit. The distance between the blasting point and the blasting machine was about 60 meter.
A digital ohmmeter was used to test the electric circuit to ensure that there was no open or short circuit.

The blastings were initiated using the capacitor blasting machine (exploder). This blasting machine was hand-activated generator that charged a bank of capacitors. When fully charged, a neon light glown and reached high potential of 1200 V. By pressing the firing button, the capacitors discharged the potential into the firing circuit and ignited the detonator and thereby an explosion occurred.

This procedure was repeated with different ANFO mixing ratios of 94:6, 90:10, 80:20, 60:40 and 50:50, keeping the absorbent and nonabsorbent surfaces at a distance of 5 m , 7m and 9 m (Table 2) from the center point.
All blasting exercises were conducted by an authorized licensed shot firer and the post blast residue on absorbent and nonabsorbent surfaces were collected and preserved as suggested by PDRM and JKM . The post blast residues collected from the ANFO mixing ratios of 94:6, 90:10 and 80:20 were analyzed in GC-MS at Chemistry Department Malaysia, PJ, Selangor and the mixing ratios of 60:40 and 50:50 were analyzed at Fire Investigation Laboratory, Fire and Rescue Department Malaysia, Kuala Terengganu, with the assistance of JKM and fire department staff as per their standard operation procedure and then recorded the findings.

Table2: Different mixing ratios of AN and FO and the interdistance between the centre blasting point and absorbent, nonabsorbent surfaces placed around with known distances.

No.	ANFO mixing ratio (by weight)	Distances between blasting point and surfaces
1	94:6	5m, 7m, 9m
2	90:10	5m, 7m, 9m
3	80:20	5m, 7m, 9m
4	60:40	5m, 7m, 9m
5	50:50	5m, 7m, 9m

Results

The controlled surface ANFO blast study indicated that the fuel oil could travel to a distance of 9m and found in the absorbent and nonabsorbent surfaces after the blast when the AN and FO mixing ratios were 94:6, 90:10 and 80:20. When the AN and FO mixing ratios were 60:40 and 50:50, the fuel oil could travel up to 5m only (Table 3) and also observed characteristic FO flash pattern in the crater (Fig 4-5), a novel finding in this controlled blast exercises. Selected mass chromatograms (Fig 6-10) and the hydrocarbon presence and retention time are presented here (Table 4-6).

Table 3: Varying ANFO mixing ratios: Detection of fuel oil in post-blast residue and formation of fuel oil flash pattern

ANFO mixing ratio (by weight)	**Soil samples collected at the seats of explosion**	**Detection of oil residue collected in post blast residues in absorbent and nonabsorbent surfaces at the distance between blasting point and absorbent , nonabsorbent surfaces (m)**		
		5	**7**	**9**
94:6	Detected FO	Detected FO	Detected FO	Detected FO
90:10	Detected FO	Detected FO	Detected FO	Detected FO
80:20	Detected FO	Detected FO	Detected FO	Detected FO
60:40	Detected FO and FO flash pattern	Detected FO	Undetected FO	Undetected FO
50:50	Detected FO and FO flash pattern	Detected FO	Undetected FO	Undetected FO

Fig 4: FO flash pattern formation the in the crater (60:40)

Fig 5: FO flash pattern formation the in the crater (50:50)

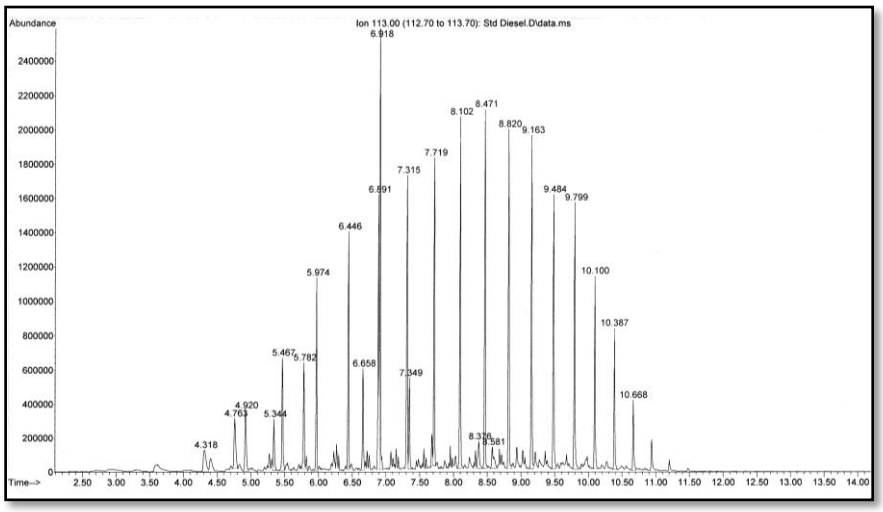

Figure 6: Mass chromatogram for m/z 113 of standard of fresh diesel fuel

Table 4: Identification of hydrocarbons present in the fresh diesel standard and its retention time (min)

No. of carbon atoms	Compound name	Retention time (min)
C12	Dodecane	4.318
C13	Tridecane	4.920
C14	Tetradecane	5.467
C15	Pentadecane	5.974
C16	Hexadecane	6.446
	Pristane	6.891
C17	Heptadecane	6.918
C18	Octadecane	7.315
	Phytane	7.349
C19	Nonadecane	7.719
C20	Eicosane	8.102
C21	Heneicosane	8.471
C22	Docosane	8.820
C23	Tricosane	9.163
C24	Tetracosane	9.484
C25	Pentacosane	9.799
C26	Hexacosane	10.100
C27	Heptacosane	10.387
C28	Octacosane	10.668

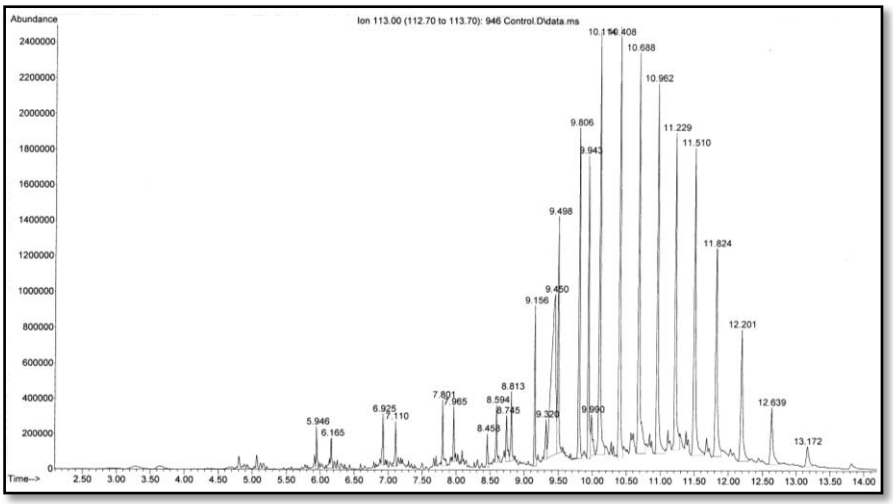

Figure 7: Mass chromatogram for m/z 113 of 94:6 soil sample collected at the seat of explosion.

Table 5: Identification of hydrocarbons present in the post blast(94:6) soil sample collected at the seat of explosion and its retention time (min)

No. of carbon atoms	Compound name	Retention time (min)
C21	Heneicosane	8.458
C22	Docosane	8.813
C23	Tricosane	9.156
C24	Tetracosane	9.498
C25	Pentacosane	9.806
C26	Hexacosane	10.114
C27	Heptacosane	10.408
C28	Octacosane	10.688

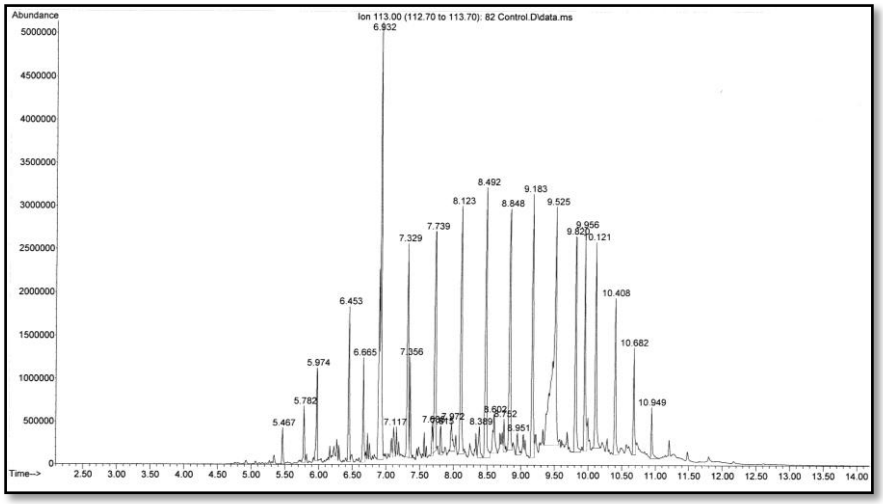

Figure 8: Mass chromatogram for m/z 113 of 80:20 soil samples collected at the seat of explosion

Table 6: Identification of hydrocarbons present in the post blast (80:20) soil sample collected at the seat of explosion and its retention time (min)

No. of carbon atoms	Compound name	Retention time (min)
C17	Heptadecane	6.932
C18	Octadecane	7.329
	Phytane	7.356
C19	Nonadecane	7.739
C20	Eicosane	8.123
C21	Heneicosane	8.492
C22	Docosane	8.848
C23	Tricosane	9.183
C24	Tetracosane	9.525
C25	Pentacosane	9.820
C26	Hexacosane	10.121
C27	Heptacosane	10.408
C28	Octacosane	10.682
C29	Nonacosane	10.949

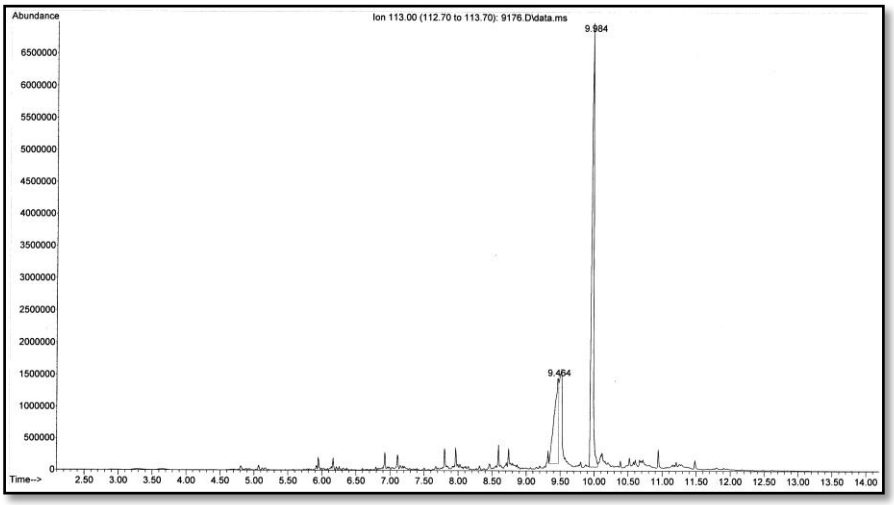

Figure 9: Mass chromatogram for m/z 113 of 90:10 dry leaf samples at 7 m distance.

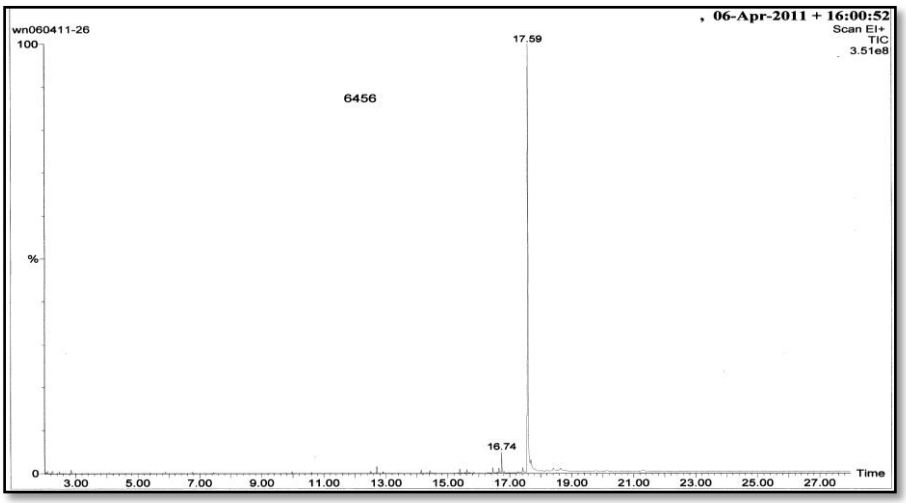

Figure 10: Mass chromatogram of 60:40 dry leaf sample at 5 m distance.

Discussion

The role of Forensic Scientists in examining explosives and their debris both in the crime scene and forensic science laboratory is largely concerned with those explosives that are illegally used. Legal or legitimate use of explosive is mainly by military and certain industries. The explosives used in industries like quarries for example, ammonium nitrate and fuel oil, are not generally termed as bombs since they are used for legitimate mining purposes only. In contrast with the above two types of explosives, there is another category generally known as Improvised Explosive Devices (IED). These are bombs manufactured illegally by miscreants and these do not conform to any specifications.

During this surface blasting exercise, the weather condition was fine with moderate temperature without rain and wind. The environmental conditions such as weather patterns, rain, and wind may be considered [10] during the blast which influence the effects of blast and the travelling distance of oil residue. The controlled ANFO surface blasting study [11] here using 500g of AN indicated that the fuel oil could travel to a distance of 9m and found in the absorbent and nonabsorbent surfaces when the ANFO mixing ratio was standard 94:6 or nearly standard proportion viz. 90:10 and 80:20. When the ANFO mixing ratios were deviated from the standard mixing ratio to improper mixing proportions viz. 60:40 and 50:50, the fuel oil could not travel beyond 5m. But invariably the post blast fuel oil residue could be detected in all the blast crater marks irrespective of the mixing proportions used for blastings. Another interesting feature observed was the formation of FO flash pattern with varying diameter. That is, the flash diameter formed from 50:50 mixing ratio is smaller than the flash diameter formed from 60:40 mixing ratio.

According to the theory of ANFO energy output, as the percentage of fuel oil increases from the standard ANFO proportion 94: 6, the energy output would decreases [12] as shown in Fig 11 and hence fuel oil could not be detected beyond 5m i.e. when the fuel oil proportions were 40 and 50. In these two fuel oil proportions, variations in size of the flash pattern were observed i.e. higher the fuel oil proportion, smaller the diameter of the fuel oil flash pattern. These findings would form a base for the crime reconstruction and be a clear lead to the forensic investigators to solve the crime in the ANFO blasting scenes.

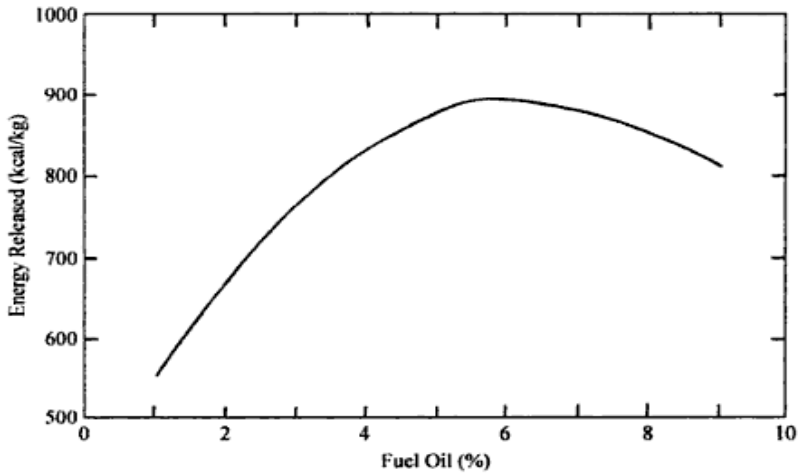

Fig11: Relative ANFO energy as a function of the percent fuel oil used

Conclusions

Further ANFO blast research is needed in future for the detailed study on fuel oil flash pattern formation in the crater for application in blast scene investigations. The environmental conditions during the blast such as weather patterns, rain, and wind should also be taken into consideration.

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Qualitative and Quantitative Detection of Rhodamine B Extracted from Different Food Items using Visible Spectrophotometry

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ABSTRACT: A study was done to detect the presence of Rhodamine-B dye quantitatively and qualitatively using Visible Spectrophotometry. Rhodamine B was found to be illegally used by sweet makers or bakers for colouring the different confectionery. The study involved the detection of Rhodamine-B quantitatively and qualitatively from street foods (Sweets and Confectioneries) collected from different cities in India. A total of 75 samples of different varieties of sweets and confectionery were collected from street food corners/ locations in Delhi, NCR region, Mumbai, Pune, Agra and Kerala. A standard control of the Rhodamine-B was used with a concentration of 20µg/ml. Samples were extracted based on organic solvent extraction using chloroform and isobutanol. The absorbance and concentration were measured using visible spectrophotometry at 503 nm. The concentrations of the extracted Rhodamine-ranged from 0.07 to 1.09 µg/mL and absorbance values were ranging from 0.08 to 1.27. Out of the total 75 sample tested, most of the 30 positive specimens were from Pune. The present study indicates that there is prevalence of Rhodamine-B use in street foods mostly in the Pune regions. The extraction method was found useful even in extracting Rhodamine-B with low concentrations. Further research is needed to determine the prevalence of Rhodamine-B from the street foods.

Keywords: Rhodamine-B , Visible Spectrophotometry, detection , street foods

Introduction

Food adulteration is defined as the process by which the quality or the nature of a given substance is reduced through the addition of a foreign or an inferior substance and the removal of a vital element [1]. More recent studies showed the use of nonpermitted colours like Rhodamine Orange II, and Auramine in street food like jilebi and coconut burfi (coconut based fudge) [2]. Another study in which 700 food items were analyzed from urban areas and 300 from rural areas showed that 93% and 95% respectively contained permitted colours viz. tartrazine, sunset yellow, ponceau 4R, carmoisine, erythrosine and brilliant blue while 7% of the food from urban and 5% from rural contained non-permitted colours [3]. The use of certain dyes has been banned, as they are found to be toxic in experimental animals.

With the liberalization of trade in India, there is a growing list of food additives and processing aids which require approval from the regulatory authorities for their use in different foodstuffs. There is an urgent need for the scientific community in India to evaluate whether these additives are necessities as they are hazardous to the Indian consumer. In India several independent studies carried out in various parts of the country have focused on adulteration of different food with added colours. The findings of most of these studies showed that a variety of food such as milk products, including ice cream, khoya (a dairy product which is made by and reducing the milk to a semi-solid stage), cottage cheese, non-milk products (sweets, savouries), legumes, miscellaneous (confectionery, soft drinks, spices, condiments, tea, flattened rice, fish, fresh vegetables and cut fruit) and spices (turmeric, chilli powder) were usually adulterated with non-permitted colours such as metanil yellow, auramine, rhodamine B, congo red, malachite green and orange II [4,5].

A market survey carried out in 236 outlets in urban areas showed that a variety of food from categories like breakfast accompaniments, beverages, sweetmeats, bakery food, savouries and confectionery contained added colours. However certain food such as spices, condiments, rusk, vegetables, savouries and a variety of cooked food preparations such as soups, noodles, gravy curries, starters, manchuria (starters made from chicken, or vegetables with cornflour and sauces), biryani (rice preparation made vegetables or chicken or lamb), ground legume flour used in preparation of savouries like sev, chegodi, boondi, finger fries, bajjis, all of which do not form a part of the PFA permitted list of specified food items, were found to contain added colours [6].

Several cases of adulteration of food with colours have been recorded. Mustard seeds were adulterated with non-permitted colour (not specified) but had conformed to the standards [7]. Metanil yellow, a non-permitted colour was a common adulterant in food like laddu, toor dal and turmeric, which could be due to its easy availability and reasonable cost [8]. Spices like chilli powder were found to contain non-permitted colours like sudan dyes [9]. Analysis of samples of sweets and confectionery collected during festivals showed the wide usage of nonpermitted colours like rhodamine to the extent of 10-95ppm, orange II (135-560ppm) and auramine (15-400 ppm) [10]. The use of permitted colours also evoked concern as they were used in excess of the statutory limit (100ppm) to an extent of 15157 mg/kg in sweetmeats and 9450µg/ml in beverages or they were used in food in which they were not permitted [11].

Rhodamine is used as a dye and as a dye laser gain medium.Rhodamine dyes are generally toxic, and are soluble in water, methanol and ethanol. Rhodamine is a banned dye as per PFA act (1954), by Government of India, because the same dye has found to be carcinogenic in human.

Different methods of detection of Rhodamine are in use in the laboratories like TLC,UV, HPLC, Paper Chromatography. Rhodamine is a banned dye as per PFA Act, 1954. Rhodamine is harmful to human as it is carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity. Rhodamine is used for food adulteration in Sweets and Confectionery.It causes health hazards in humans who consume it. Examination for this is been done for the detection of Rhodamine from Food Stuffs in Forensic Toxicology Laboratories and Food Adulteration Laboratories.

The objective of the present study was to detect the presence of Rhodamine-B dye quantitatively and qualitatively from street foods using visible spectrophotometry. Since Rhodamine B is a legally banned dye in India as per PFA Act 1954 and as per subsequent amendment in 2004, it has been used by sweet makers or bakers for coloring the different confectionery, the result of the present study would give enough scope to further explore the possibilities of extraction of Rhodamine and its detection through qualitative and quantitative analysis. The present study has given more emphasis on the detection of Rhodamine-B extracted from street foods (Sweets and Confectionery) collected from different regions in India as it has been found that the use of Rhodamine-B in sweets and confectionery were more predominant in the street foods available in different metro Cities in India.

Sample size

In total 75 samples of different varieties of sweets and confectionery were collected from street food corners/ locations from Delhi, NCR region, Mumbai.

Criteria for Sample Collection

Samples were collected on the basis of its color like pink, rose, and red. Only sweets, squash, syrups were collected. The samples were collected from different regions in India, Pune, Agra and Cochin. As control sample 2mg of Rhodamine B was used.

Materials and method

Samples of street foods (sweets, squash, syrups) control of Rhodamine-B, chloroform, Chloroform and Isobutanol, separating funnel, distilled water, water bath, visible spectrophotometer (UV-VIS Spectrophotometer(Elco-SL-150).

The standard control of Rhodamine-B was prepared by dissolving 2 mg of Rhodamine B in 100 ml of chloroform to obtain 20 µg/ml. The organic solvents used for the extraction of Rhodamine B were chloroform and Isobutanol. The solvents were mixed in a ratio 8:2 and mixed with water. The samples of sweets were ground or macerated to an aqueous slurry and was shaken with the extraction solvent i.e. Chloroform: isobutanol for 30-45 mins. The squash, syrups were shaken with the extraction solvent in the similar way. The contents were filtered and residual slurry were again extracted twice with extraction solvent mixture. The filtered fractions were combined and taken in to separating funnel and into the same 10-15 ml distilled water was added and shaken vigorously. Chloroform layer was collected and the process was repeated. The chloroform layers were combined and the same was concentrated to 10 ml by evaporating on water bath and the sample was subjected for visible spectrophotometric analysis.

Results and discussion

Out of 75 samples experimented 60 samples showed positive results and among 60 samples the maximum positive results were showed by the samples from Pune. Quantitative and qualitative estimation of Rhodamine was performed by comparing the absorbance value of Rhodamine at a particular wavelength of maximum absorption i.e. 503 nm. The absorbance of control sample formed the basis of comparison with the samples, which consisted of randomly sampled food substances. Absorbance values of control as well as 60 test samples shows that the concentration of Rhodamine-B in the spurious food samples

collected from street were differing. This finding substantiates the results of previous study Pune [12]. The present study established that there is a prevalence of Rhodamine in street foods mostly in the regions like Pune and the study is giving a scope for further research in same field. The absorbances and concentrations were measured using visible spectrophotometry at 503 nm. The concentration of the extracted Rhodamine B ranged from 0.071 to 1.09µg/ml and the absorbance ranged from 0.084 to 1.27.

Statistical analysis was done and the t value was found to be 8.08 and p value was found to be 0.01.

Table 1: Absorbance and Concentration of Control and samples

Samples no.	Absorbance	Sample Concentration (µg/ml)	Difference of absorbsnce	% difference of absorbance
1	0.973	0.837	0.131	11.7
2	1.104	0.95	0.009	0.808
3	0.783	0.67	0.33	29.6
4	0.972	0.833	0.141	74.8
5	Nil	Nil	Nil	Nil
6	1.104	0.935	0.178	100.8
7	0.0845	0.071	1.028	131.71
8	0.956	0.81	0.157	116.4
9	0.904	0.76	0.209	123.1
10	Nil	Nil	Nil	Nil
11	1.245	1.05	-0.132	-11.1
12	1.109	0.94	0.004	99.6
13	Nil	Nil	Nil	Nil
14	0.864	0.73	0.249	77.6
15	0.894	0.75	0.219	124.4
16	Nil	Nil	Nil	Nil
17	1.09	0.92	0.023	102.1
18	1.001	0.84	0.112	111.18
19	Nil	Nil	Nil	Nil
20	0.978	0.82	0.135	113.8
21	0.995	0.84	0.118	111.8
22	Nil	Nil	Nil	Nil
23	0.973	0.82	0.14	114.3
24	Nil	Nil	Nil	Nil
25	0.94	0.79	0.173	115.9
26	Nil	Nil	Nil	Nil
27	1.11	0.94	0.003	100.2
28	0.895	0.75	0.218	124.3
29	0.675	0.57	0.438	164.8
30	0.564	0.47	0.54	197.3
31	nil	Nil	Nil	Nil
32	0.76	0.64	0.353	146.44
33	0.756	0.64	0.357	147.2
34	nil	Nil	Nil	Nil
35	1.273	1.09	-0.16	114.3
36	nil	Nil	Nil	Nil
37	1.113	0.94	0	100
38	nil	Nil	Nil	Nil
39	nil	Nil	Nil	Nil
40	1.041	0.88	0.072	106.9
41	nil	Nil	Nil	Nil
42	0.864	0.73	0.249	128.8
43	1.00	0.84	0.113	89.84
44	Nil	Nil	Nil	Nil

45	1.113	0.943	0	100
46	0.97	0.95	0.143	12.8
47	1.1	0.99	0.123	11.05
48	0.78	1	0.33	29.6
49	0.97	0.91	0.143	12.8
50	1.1	1.67	0.013	1.16
51	0.08	1.2	1.046	93.9
52	0.95	0.96	0.163	14.6
53	0.9	0.99	0.213	19.13
54	1.24	1.57	-0.127	-11.4
55	1.1	1.43	0.013	1.16
56	0.86	1.32	0.253	22.7
57	0.89	1	0.223	20.03
58	1.09	1.3	0.023	2.066
59	0.97	0.98	0.143	12.8
60	0.99	1.45	0.123	11.05
61	0.94	1.32	0.173	15.54
62	1.11	1.57	0.003	0.26
63	0.89	0.96	0.223	20.03
64	0.67	1.42	0.443	39.8
65	0.56	1.2	0.553	49.6
66	0.76	1.02	0.353	31.7
67	0.75	0.99	0.363	32.6
68	1.23	1.07	-0.117	-10.5
69	1.11	1.6	0.003	0.269
70	1.04	1.64	0.073	6.558
71	0.86	1.32	0.253	22.7
72	1	1.52	0.113	10.15
73	0.86	1.43	0.253	22.7
74	1	1.07	0.113	10.15
75	1.11	1.09	0.003	0.269

Table 2: Mean,Standard deviation and t&p values

MEAN ABSORBANCE	STANDARD DEVIATION	t VALUE	P VALUE
0.85	0.23	8.08	Less than 0.01
1.25	0.25		

For the qualitative and the quantitative estimation of Rhodamine-B extracted from the food stuffs using the methodology adopted in this study , one has to consider a proper method of extraction as it has been found that the Rhodamine-B is found to be relatively less in concentration when conventional techniques in extraction were used which would definitely affect the results of Rhodamine detection.

The present Visible Spectrophotometric qualitative and quantitative detection of the presence of Rhodamine-B extracted from the food stuffs using the methodology adopted would definitely give an insight to the forensic scientific community to further explore the possibilities of detection of the same using the methodologies in similar lines.

Conclusion

For the detection of Rhodamine-B from suspected samples collected from street foods, one has to be considering the standardized methodology adopted to detect Rhodamine B. Rhodamine B is commonly and illegally used for coloring of food which cause a lot of health hazards. Absorbance value of control as well as 30 test sample shows that the concentration of Rhodamine in the spurious food Samples from street detected were differing. Rhodamine B is a carcinogenic substance, mutagen, tumorigen Continuous consumption of foods containing Rhodamine cause many ill effects and even damage body organs as well. The toxicity studies of Rhodamine shows that it is causing toxicity to

humans, including carcinogenicity, reproductive and developmental toxicity, neurotoxicity, and acute toxicity.

Since Rhodamine-B was detected from samples and hence the sample size was lesser ,it gives a scope for further research to explore the probability of detection of Rhodamine in such foods collected from street food corners considering more number of samples so that the prevalence of the use of Rhodamine in street foods in different regions in India could be assessed and also the types of food which are adulterated .The results of the present study is indicating and giving an alarming signal with regard to street food adulteration with Rhodamine, which are consumed by common people thus causing health hazards.

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Amino Acid Racemization from Tooth for Age Estimation- An Overview

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ABSTRACT: Age estimation constitutes an important factor in identification of individual in forensic odontology. Out of the various methods used, Amino acid racemization (AAR) is the most reliable one. AAR occurs in tissues with low metabolic turn over. As age advances L-amino acid change to D-amino acid, and their ratio represents age. Amino acid racemization method has a historical background of over three decades. Since most of the methods used in forensic odontology age estimation were based on structural changes of teeth, racemization becomes the newer method by its chemical approach. In general racemization of amino acid follows a first order reversible rate law; L- amino acid K_L/K_D D- amino acid where K_L and K_D are the first order rate constant of the interconversion of L and D amino acid enantiomers. Amino acid from dentin as well as from enamel is tried for racemization study. Several studies were conducted in this field and most of it had an age prediction range of less than ± 3 years. Advantage of using this method is that minute amount of tooth sample is well enough to perform this technique. This paper highlights an overall view of AAR method with relates to age.

Key words: Amino Acid Racemization, age estimation, forensic odontology

Introduction

Age can be estimated in children and in adolescents by means of development and eruption of deciduous and permanent teeth up to 14 years. For most age estimation methods the developing teeth are subjectively assessed on radiographs. After the age of 14, the third molar is the only remaining tooth that is still developing and consequently dental age estimation methods have to rely on the development of this tooth until the age of 20. After this period age determination is mainly done by visual examination, radiographic methods [1, 2] of structural changes in teeth and by means of chemical methods [3].

About 37 years back it was found that the extent of AAR could be used for dating of various biological materials. Later it was found that chronological age of an individual could also be estimated with a reasonably good precision [4]. At a temperature of 25 °C, it would take 100,000 years for all L-forms of amino acids present in living tissues to undergo complete racemization to the D-amino acid form [5]. This chemical method of age determination uses teeth from living as well as from deceased individuals. A gradual transformation of L-aspartic acid into its D-form (racemization) occurs during life in metabolically inactive tissues (eg. Tooth enamel, dentin, white brain matter and lens of the eye) and the ratio of the two forms is an expression of age [6].

AAR based on age-dependent, nonenzymtic changes of L- form amino acid to D-form amino acid is considered to be one of the most reliable and accurate methods in estimation of age of an adult individual [7].

Review

Racemization of aspartic acid proceeds throughout lifetime and also after death, but probably at a reduced rate as a result of a presumed reduction in ambient temperature. In fresh cadavers or putrefied remains racemization of aspartic acid is applicable as long s the post-mortem interval does not exceed a few decades [8].

D/L ratio can be assessed by using either gas chromatography or by high performance liquid chromatography. Vanden Oetelaar [9] compared the separation efficiency of both and found that HPLC is preferred because of its higher reproducibility and convenience. But recent research point out that both method have its own advantages and draw backs [9-18]. The method has high sensitivity, contamination with proteins from other sources, such as blood, pulp or periodontal tissues may significantly increase the relative amount of the L- form, giving a too low estimate of the age. Also newly formed secondary dentin may decrease the relative amount of the D-form. Similarly contaminated with bacteria with D-form aspartic acid in their cell wall may cause an apparent too high age. The rate of racemization of amino acid in dentin in the living body is mainly determined by temperature, pH and water content [6].

As the post mortem temperature of cadavers rapidly reach the temperature of the preserving environment,

the racemization rate decreases. But the age estimation on cadavers indicted that post mortem preservation of upto 10 years had a negligible effect on the values estimated for the age of individuals at death [10].

In general racemization of amino acid follows a first order reversible rate law: L- amino acid K_L/K_D D-amino acid Where K_L and K_D are the first order rate constant of the interconversion of L and D amino acid enantiomers. If the ratios of the abundance of the D and L amino acid and K_L are known, the age of the tooth can be calculated by statistical regression analysis [5].

Many studies were done on age estimation using racemization of amino acids [5,6,10,11]. T. Ogino found that unerupted teeth with normal crown shape and size can be used for age estimation [5]. Susumn Ohtani showed not only total amino acid in dentin, but also of its fractionated and extracted substances, can lead to higher reliability in age assessment [6]. The author also showed that deciduous teeth can also be estimated by racemization technique [6]. Out of various aminoacids, aspartic acid was highly correlated with age [10].

There is broad consensus among experts about the best suited age estimation methods as described in the recommendations of the Study Group on Forensic Age Diagnostics [12]. Among the reliable methods, racemization of aspartic acid in dentin ranks first. Available reference works compiled to date result in an average regressive error of estimate of 2.1 years [12].

Shi-Jiang Fu et.al compared the effectiveness of GC and HPLC in separating aspartic acid and HPLC was reported to be better [13]. The highlights of the material and methods in AAR technique as mentioned by Shi-Jiang Fu et.al is as in (i) – (iv) below.

i. *Apparatus*
The HPLC system consisted of a 510 pump and 420 fluorescence detector (Waters), reverse phase column (250 x 4.6 mm).

ii. *Chemicals*
The chemicals used were I> and L-aspartic acids (BDH), N-acety-L-cysteine (NAC. SIGMA), O-phthalaldehyde (OPA. SIGMA), HPLC-grade water and methanol. All other chemicals were reagent grade.

iii. Sampling teeth
The first premolar was used in every case. Experimental teeth were extracted from cadavers and stored in 10% formalin. Before use, all the teeth were washed under running water overnight and then allowed to dry naturally.

iv. *Sample preparation*
After storage, the tooth roots were removed, and the crowns were ground with a mortar and pestle. Dentin fragments (-40 mg) were separated by hand under ultraviolet light (they fluoresce and enamel does not). The dentin fragments were cleaned by ultrasonication in double-distilled water and dilute HCI, sealed in tubes, and hydrolyzed with 6 mol/L HCI for 6 h at 100°C. The hydrolyzates were dried under a flow of nitrogen gas, and desalted on a strong-acidity exchange resin. Amino acids were eluted with 2 mol/l ammonia solution, and specimens were once again dried by nitrogen gas, and I ml of double distilled water was added to the specimens.

v. Preparation of the OPA-NAC reagent
OPA (8.0 rag) was dissolved in 600 t~l of methanol. The following were then added in the order indicated: 500 #1 of 0.4 mol/1 Na borate (pH 9.4); 800/~1 of distilled water; and 120/zl of 1.0 mol/l NAC. The OPA-NAC reagent was stored at 4°C.

vi. Derivatization of amino acids for HPLC
Derivatization was accomplished by mixing 10 ~1 of prepared sample solution with 20 t~l of OPA-NAC reagent in a small test tube. After 2.5 min, 200 ttl of 50 mmol Na acetate (pH 5.2) was added, then 10 ~tl of the solution was taken for direct injection into the HPLC system. HPLC operating conditions: mobile phase was 92% of 50 mmol acetate (pH 5.7) and 8% of methanol; the fluorescence detector was equipped with a 300-400 nm excitation filter and a 400-700 nm emission filter; the flow rate was 1.0 ml/min

Ohtani et al [14] suggested that in elderly individuals racemization in teeth that have been situated deep in the oral cavity for a long time (and thus are exposed to higher ambient temperatures) are more influenced by the environment than by the period of tooth formation. Criteria for selecting tooth for racemization technique is effecting the age of the individual. The types of teeth best suited for racemization analysis are single rooted teeth such as mandibular incisors or mandibular premolars[15]. In these teeth, all the dentin can be easily collected, and test results have shown that analysis using whole dentin yields a more accurate age estimate than analysis using only part of the dentin [15].

Dentin is not a homogeneous substance. Initially primary dentin is formed at the crown potion followed by root portion. Throughout the life secondary and tertiary dentin are formed, their age will be definitely lower than primary dentin. Dentin formation is different in different teeth as the eruption dates [16]. Approximately 8-10 years are needed from start to completion for dentin; hence

degree of racemization may differ at different parts of teeth [16]. Degree of aspartic acid racemization (AAR) is expected to be higher in the earliest formed tooth like 1st molar. On contrary, 2nd molar shows high degree of racemization. It may be because molar teeth are deeper in the oral cavity. So, the teeth of older individual are more affected by environmental cause than the time since the moment of formation. AAR is highest in second molar and decrease in the following arder >1st molar> 2nd premolar> central incisor> 1st premolar> lateral incisor> canine [14].

There is no variation of AAR of same teeth in right or left sides of jaw. There is a higher racemization rate in the lingual section of teeth crown than buccal section, because the lingual side may be exposed to high temperature in the mouth. Whereas no changes in root section of dentin were noticed [15,17]. It was suggested that AAR is more in crown and low in root apical portion in young individual, whereas it is affected in elderly as there is prolonged period of time the tooth apex remains there [18].

Dentin consists of approximately 91% of acid insoluble fraction (collagen) and 9% acid soluble fraction (non-collagen) [19, 20]. AAR is rapid in non-collagen protein but slow in collagen protein. Racemization of whole dentin is almost the same as racemization of collagen portion, since majority of dentin consist of collagen. Central incisor showed better correlation [18]. Standard specimen is prepared from D&L aspartic acids, which can be substituted for control teeth. This will be used as standard specimen in other laboratories as well; hence racemization can be measured with reproducibility [21]. It is preferred to do AAR technique in known ages of individual until the standard error of ± 3 years are obtained before starting the procedure in unknown age group [22].

Difference in specimen of dentin and difference in analytical methods have made the different results in various published studies. AAR is strongly influenced by the temperature [18]. Using a whole dentin is preferred to get an accurate result. And preferred tooth is ideally being incisors or premolars since they are single rooted and maximum dentin is easily attainable. HPLC coupled with florescence detection have improved the results of AAR [18]. Racemization reaction progresses more rapidly in the root than in the crown [23]. The effects of various experimental conditions [24], heating [25] and post-mortem changes [26] were also studied on the racemization reaction. Cases with prolonged post-mortem intervals (beyond decades), human remains with advanced degradation and burnt bodies should be treated with caution [27].

The fact is that age estimation by aspartic acid racemization from teeth requires specific technical background which, in general, might not be available in all forensic laboratories and a great deal of standardization. Therefore, suggestions for an international standardization and recommendations for methodological approach were published [28]. The use of internal standards prevents experimental errors occurring from sample preparation, derivatization and separation. It is recommended for techniques where accuracy and reproducibility are of major concern. S. Ritz [23] concluded that the application of D-methionine as internal standard was proposed since it is properly separated as well as nearly eluted to Asp and provided calibration curves with excellent linearity. Once the standards are prepared it can be used for few months.

Griffin's [29] study showed a promising idea of using enamel as a source for AAR. The proteins of mature enamel are processed during enamel maturation by proteolytic enzymes to form low molecular weight peptides. During enamel formation the enamel proteins become entrapped in the growing crystalline structure of the enamel. As a result, enamel provides a much better source of intra-crystalline proteins than dentine. Moreover, enamel is much more resistant to change in the burial environment [30]. AAR by enamel sample is very rarely used as the levels of protein are so low that it presents a real analytical challenge [4, 30] and the correlation between racemization and age is not as strong as in dentine [17]. Previous work required large amounts of enamel, sometimes requiring destruction of an entire tooth for a single analysis. However, the introduction of reverse phase-high pressure liquid chromatography (RP-HPLC) with fluorescence detection has made it feasible to determine the extent of racemization of much smaller quantities of amino acids than had previously been possible. RPHPLC can detect amino acids at quantities as low as 0.9 pg [31]. The presence of dental caries in the tooth analyzed only has a small effect on the racemization of the tooth, and thus even carious teeth could be used in age estimation, albeit with slightly larger confidence intervals. Amino acid racemization in enamel can be used in cases where minimal sample destruction is required.

Jir et.al [32] made a study using non-collagenous protein from dentin for assessment of age. This method exploits the characteristic of staphylococcal protease V8, which specifically splits peptide bonds where Lglutamic or L-aspartic acid participate. Those peptide bonds where D-aspartic acid is present remain unsplit because of the stereospecifity of enzymes. In accordance with expectations, fewer peptide bonds are split by this protease at more advanced ages and larger peptide fragments are thus formed due to the higher content of D-amino acid residues in the proteins of older people. The samples of acid-extracted non-collagenous proteins from

dentin were separated using high performance liquid chromatography after enzymatic hydrolysis. A peak with a retention time of 45.3 min was chosen and his enlarging area showed a linear correlation with increasing age. Although the linear correlation with age was proved, the scattering of values decreases the usefulness of the proposed method for age estimation. Some of the present drawbacks may be eliminated by further research.

Conclusion

AAR is still the best method in estimation of age of an individual. Whole root dentin is used mostly as the source of amino acid. The best teeth selected are of single rooted tooth. Non-collagenous dentin is also tried for age assessment method, but needs further studies for its usefulness. Racemization from enamel is the new approach with a promising result, especially if the sample is of minute amount. Internal standard developed for the sampling stage improved the accuracy of AAR method.

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Macroscopic Examination of Morphological Features of Propellant Powders Prevalence in Malaysia

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ABSTRACT: Propellant powders act as fuel to propel the bullet towards the target. The combustion of powders produces a large amount of gas, increases the pressure of the casing and detaches the projectile towards the target. The shape and geometry of powder particles, as well as the chemical composition vary among different ammunition types, affecting the burning rate of these propellant powders. Here, the morphological features of propellant powders *i.e.* shape, colour, dimensions and weight were examined. Each criterion was compared among the ammunition types. Results show only one single type of particle shape in all the ammunition analysed. Colour examination shows that one of the eight sample types analysed in triplicates had a mixture of two colours. The size and the weight of the powder particles correlate with each other where greater size particles have heavier weight and vice versa. Comparison of the morphological features allows the propellant powders to be distinguished from each other through the macroscopic examination of propellant powders.

Keywords: Macroscopic examination, morphological features, propellant powders

Introduction

Propellant powders in cartridge case act as fuel to propel the bullet towards the target. The combustion and burning of the powders produce great amount of gas and increase the pressure rapidly inside the casing, which subsequently detach the bullet [1-4]. Smokeless powders are more commonly used nowadays compared to black powders [3-5]. Nitrocellulose forms the major component in smokeless powders. Nitroglycerine is additionally found in double base smokeless powders while nitroguanidine is also added in triple base smokeless powder. Besides explosive materials, additives such as stabilisers, placticisers, inhibitors, coolants, moderants and lubricants are added to the composition, giving a specific formula for the propellant powders [3, 6]. The formula of each propellant varies on the basis of ballistic performance and stability characteristics [7]. The size and shape of the propellant particles also undergo optimisation by the manufacturer for desired performance of the ammunition [8].

Based on burning rate, propellant powders can be classified in three categories, namely regressive, neutral and progressive burning powders [9]. The burning surface area decreases continuously along with the consumption of propellant in the regressive powders. Generally, propellant powders in the form of flakes, or spherical and cylindrical in shapes give this characteristic. Progressive burning powders continuously increase the surface of burning as the grains burn. Examples of these powders are multi-perforated and rosette shaped powders. Single perforated grains are categorized as neutral burning powders with the burning surface area remains approximately constant through the burning process [9]. Additional to the shape of the grains, the geometry of the powder grains and chemical treatments of the powders also affect the burning rate. Principally, smaller grains burn faster as compared to larger ones due to the exposure of greater surface area [9]. Figure 1 shows some examples of the shape of propellant powders.

Evidence must be preserved and analysed with care in all cases, as it could be the only trace left after a crime is committed and usually could not be further recovered in most circumstances. Examination of evidence always starts with non-destructive to destructive and from general to particular in the law of forensic science. During the burning process, the propellant powders in a cartridge are not totally converted into burning products, but part of them will remain unburned [4]. Besides gunshot residues, types of gunpowder particles present (e.g., flake, disc, ball and flattened ball.) at the scene on contact surface could provide useful information during examination for use in subsequent testing and interpretation. In such cases, morphological features of propellant powders shall be noted before any further examination. In this study, morphological features of propellant powders, *i.e.* shape, colour, dimensions and the weight were studied and compared with the aim to distinguish different propellant powders of different batches or manufacturers.

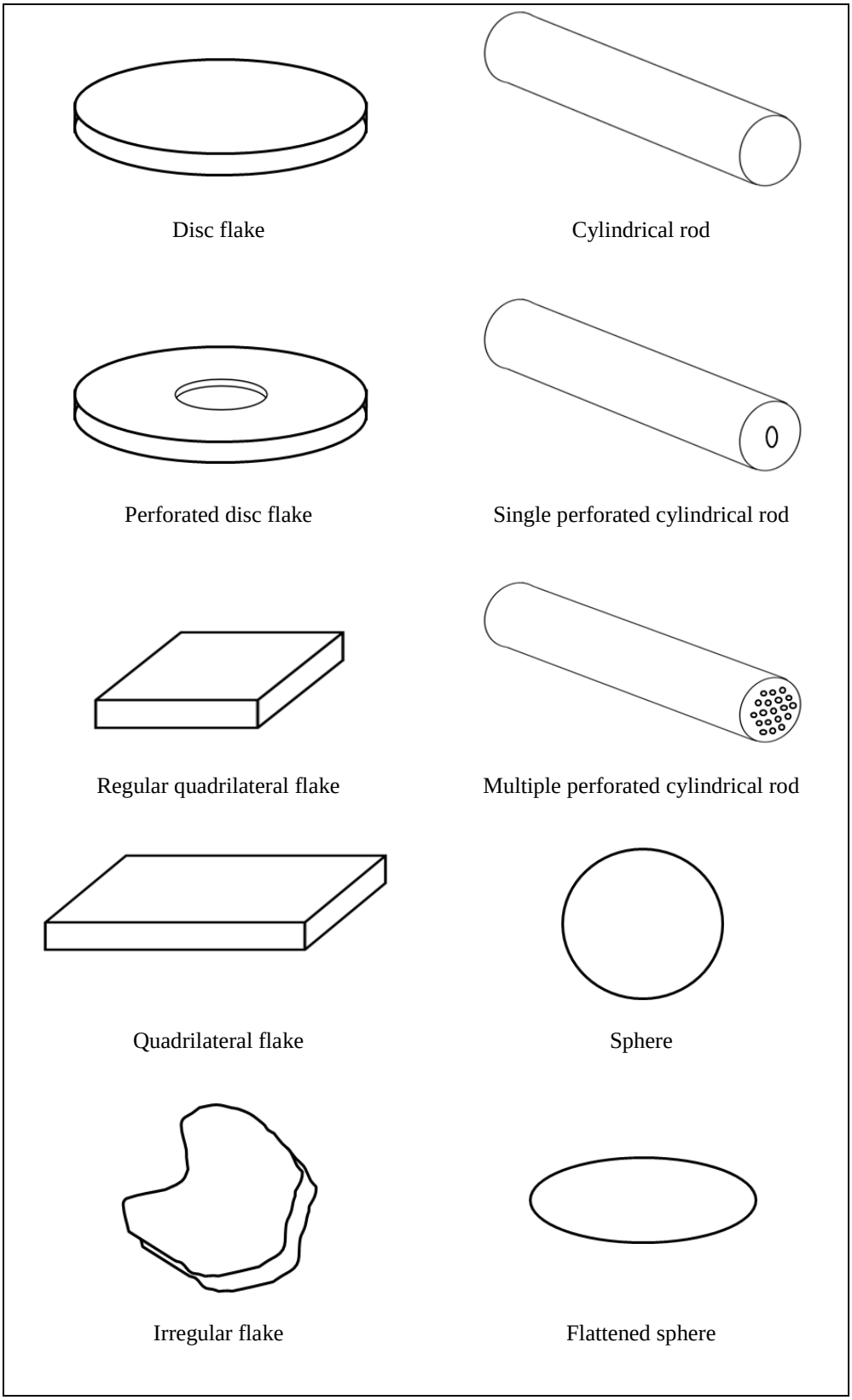


Figure 1: Shapes of unfired propellant powders [9]

Materials and method

Propellant Powders

Propellant powders from six ammunition types were supplied by Royal Malaysia Police Forensic Laboratory and SME Ordnance Ltd., *i.e.* SME 9 mm and SME .38 SPL (Malaysia), Winchester 9 mm (WCC, USA), Giulio Fiocchi-Leccon 9 mm (G.F.L, Italy), Sellier & Bellot 9 mm Br.C and 9×19 (S&B, Prague, Czech Republic). A Kinetic™ Bullet Puller (Quinetics, USA) was used to remove the bullets from the cartridges. Propellant powders were collected in 10 mL clear glass headspace vials (Supelco, Malaysia), sealed and labeled.

Microscopic Examination

A Leica Microscope MZ 16 (Switzerland) equipped with a digital camera (Leica FC 290) was used. Transmitted light base (Rottermann, TL RC 1) supplied the light source during the observation. The microscopic system was supported by Leica Application Suite software for photo capturing. Vernier caliper was used for measuring the dimensions of the propellant powders to the nearest 0.01 mm. An analytical balance (Dragon 204, Mettler Toledo) was used for weighing the samples, to the nearest 0.01 mg.

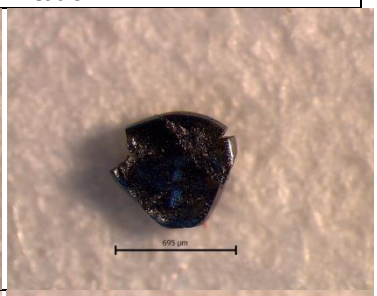
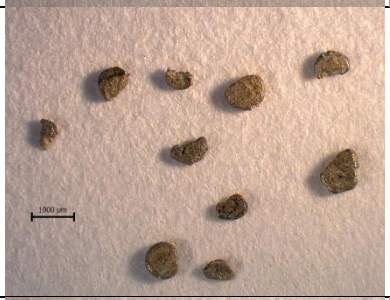
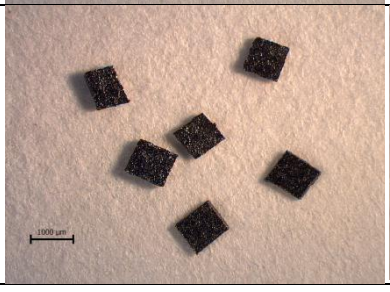
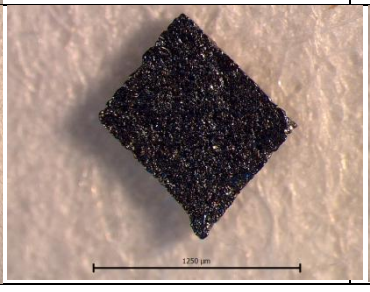
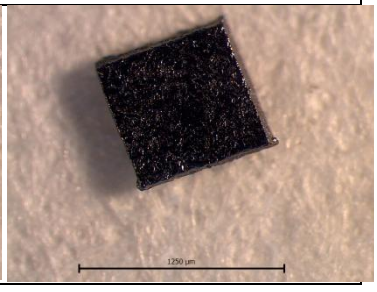
The propellant particles were magnified up to 40X during particle shape and colour examination. Photographs of each propellant particle were captured and saved as TIFF files. The dimensions of the particle were optically measured. From each propellant powders collected, seven individual particles were randomly chosen for measurement. A total of three samples from every ammunition types were examined. Mean measurements were reported with standard deviation. The intra and inter-variability of every propellant powders for different ammunition types were also analysed.

Results and discussion

Shape of the propellant powders

Among the ammunition types analysed, only one single type of particle shape was present in each sample. The shapes of powders from each ammunition type were captured in 10× and 40× magnifications (Table 1).

Table 1: Particle shape of every propellant powders studied in 10 × and 40 × magnifications

Ammunition	10 X magnification	40 X magnification	
SME (9 mm)			
WCC (9 mm)			
GFL (9 mm)			

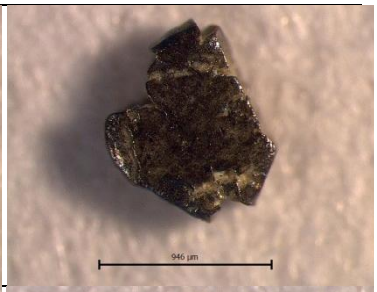
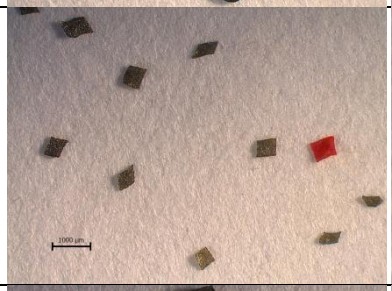
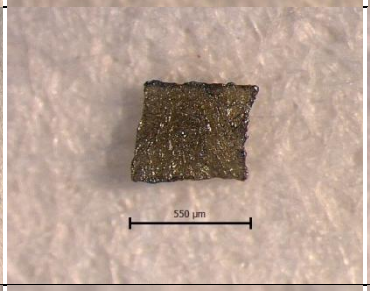
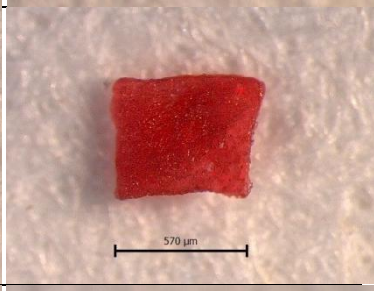
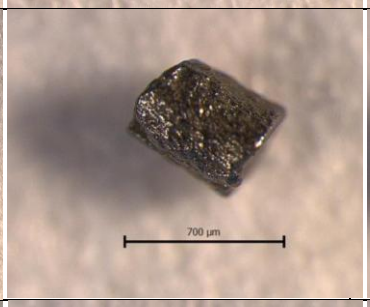
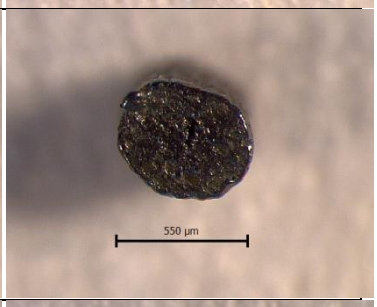
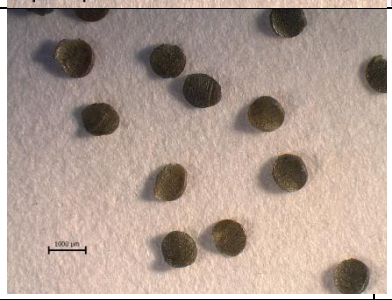
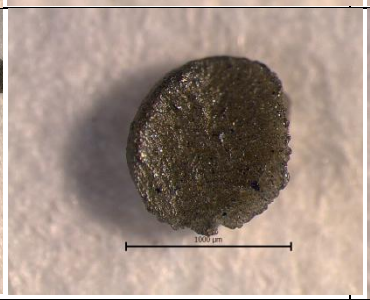
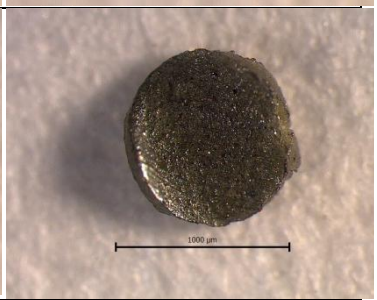
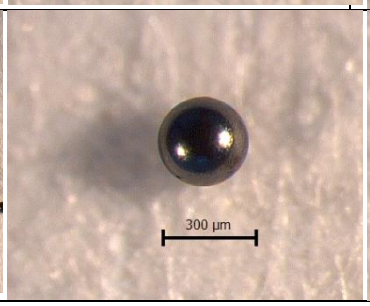
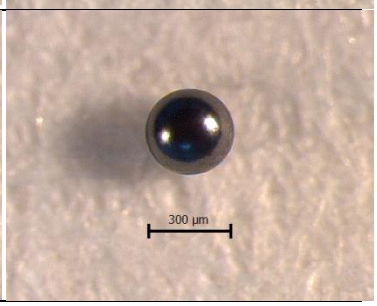
S&B (9 mm Br.C)			
S&B (9 × 19)			
			
SME (.38 SPL)			
			

Table 2 summarises the particle shapes, colours, dimensions, and the weights of every ammunition types examined in this work. Irregular flakes were found common in propellant powders, including the SME 9 mm, WCC 9 mm and S&B 9 mm Br.C. Note that irregular flakes refer to particles which do not pose any specific shape or configuration. Configurations such as cylindrical rod, sphere, disc and quadrilateral flakes were also observed.

Table 2: Particle type, colour, dimensions and weight of ammunition

Ammunition	Particle type	Colour	Dimensions			Weight (mg)
			Diameter (mm)	Width (mm)	Length (mm)	
SME (9 mm)	Irregular flakes	Shiny black	0.802 ± 0.179	-	-	0.125 ± 0.036
WCC (9 mm)	Irregular flakes	Light brown	1.030 ± 0.148	-	-	0.348 ± 0.060
GFL (9 mm)	Regular quadrilateral flakes	Shiny black	-	1.021 ± 0.040	1.025 ± 0.032	0.428 ± 0.115
S&B (9 mm Br.C)	Irregular flakes	Shiny black	0.981 ± 0.097	-	-	0.130 ± 0.033
S&B (9 × 19)	Cylindrical rod	Grey	0.695 ± 0.023	-	0.986 ± 0.245	0.374 ± 0.089
	Parallelogram flakes	Dark grey	-	0.610 ± 0.089	0.608 ± 0.083	0.039 ± 0.012
		Red	-	0.538 ± 0.053	0.549 ± 0.066	0.027 ± 0.007
SME (.38 SPL)	Disc flakes	Dark brown	1.307 ± 0.041	-	-	0.250 ± 0.021
	Sphere	Shiny black	0.388 ± 0.020	-	-	0.034 ± 0.006

Among the samples examined, two different shapes were found in SME (.38 SPL) and S&B (9 × 19) ammunition types. It was noted that these types were manufactured in different years. For instance, in SME .38 SPL ammunition types, spherical shape particles were observed in the powders collected from manufacturing year coded "02" whereas disc flakes were observed in powders from manufacturing year coded "04". In S&B 9 × 19 ammunition types, two different shapes i.e. cylindrical rods and parallelogram flakes were observed for two samples manufactured in different years. The head stamp of the cartridges could provide information to explain the difference on the particle types due to different batches or years of manufacture. In brief, examination on the shape of the propellant powders provides useful information to differentiate propellants into different groups thus aiding the subsequent identification of propellants.

Colour of the propellant powders

One of the eight sample types (all in triplicates) analysed showed a mixture of two colours. In S&B 9 mm Br.C ammunition type, majority of the parallelogram particles were dark grey but a few red flakes were observed giving a unique feature for this type of ammunition. This is an example that the variation of colours in the powder enabled them to be readily differentiated from others.

Size of the propellant powders

The size of the powders varied according to their shapes. The diameter for propellant powders with disc flakes or spherical shape was reported. Both diameter and length were reported in cylindrical shape particles, while the width and length measurements were reported for quadrilateral flakes.

In general, the diameters of the particles were between 0.6 mm and 1.4 mm. The length of cylindrical rod particles ranged from 0.7 mm to 1.3 mm. Intra-variability of the particles from the same cartridge were observed in all types of ammunition, especially in S&B 9 × 19 powders of cylindrical rod configuration with relatively greater length being observed. The insertion and compression of the powders into the confined space of a cartridge case during manufacturing process could cause deformity or breakage of these particles. However, this intra-variability of particle size did not affect the differentiation of ammunition among types in our case as this phenomenon was seen in all the samples from the same ammunition type.

Weight of the propellant powders

The weight of propellant particles was highly dependent on the size of the particles. Smaller size particles weigh lighter than bigger ones. Cylindrical rod and quadrilateral flakes were found to be heavier than the disc flakes and spherical shape particles.

Discussion

In comparison to the variability of size and weight, intra-variability with regard to the shape and colour was very low for powders obtained from a single cartridge, and was found to be extremely low among the propellant powders from the same ammunition

type. The manufacturing process of ammunition could explain these observations since the formula of propellant powders was determined upon careful research and extensive tests to reach the intended performance intended. Consequently, frequent changes in a formula is unlikely unless with specific requirements from the users.

Stringent quality assurance during the production of propellant powders also ensures that materials used were of same quality with fairly uniform physical appearance. Nonetheless, slight deviation in size and weight is still unavoidable during production. It is important to note that the same batch of powders for the same batch of ammunition leads to low inter-variability of propellant powders among the same ammunition types, especially from the same lot. Therefore, during forensic examination, similar physical appearance or chemical composition from a same box of ammunition could be expected.

Examination of morphological features provides useful information during the preliminary examination for use in subsequent testing. Identification of propellant powders in evidential materials can provide useful information to link to the types ammunition used especially when both the cartridge and projectile are absent. Although the chemical composition of propellant powders was the target of interest of forensic investigation, the morphological features should be carefully noted before any chemical tests are attempted.

Conclusion

Propellant powders showed low intra-variability in shape and colour, compared to their size and weight. Relatively low inter-variability was observed in the morphological features of propellant powders from all types of ammunition examined. Macroscopic examination of these propellant powders suggests the possibility of distinguishing different ammunition types in a simpler and quicker manner.

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A Validity Study of Malay-translated Version of Perceived Stress Scale

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ABSTRACT: In Malaysia, no local psychometric instrument to perceive stress is available. The aim of this study is to translate the Perceived Stress Scale (PSS) in the national language of Malaysia, and validate the Malay-translated versions of the PSS. The validation process was conducted among female prisoners to enable use of the Malay-translated PSS among prison population, in order to perceive stress and provide recommendation for rehabilitation. A cross-sectional study was designed. Ninety female prisoners were purposively selected as the participants. A back-to-back translation was done followed by confirmatory factor analysis and reliability testing. The Malay-translated instrument was retested among 40 participants after one-week interval. As the Malay-translated version of the instrument was found appropriate for factor analysis through preliminary analysis, the factor structure was found comparable to the original versions. The Cronbach's alpha coefficient was acceptable (α = .64) with high total test-retest reliability (R=.72). The study concluded that the Malay-translated version of the PSS was found to be comparable to the original version and to previous studies, and therefore is valid and reliable to be used in identifying stress among Malaysian prison population.

Keywords: Perceived-Stress scale, female prisoner, validity, reliability.

Introduction

Stress is one of the most assessed life experiences among people worldwide. Stress has been related to many life factors such as health status, the well-being as well as diseases [1-5]. The use of psychometric instrument to measure stress is especially important. Early detection of stress may prevent negative consequences and provide opportunities for treatment and rehabilitation. In Malaysia, published study on stress among prisoners is so far not available since there is no local psychometric instrument to perceive stress. Specific study on stress among prisoners is important to enable effective treatment and rehabilitation in prison. To enable such study, specific instrument to measure stress is needed, and the instrument itself needs to be adequate and reliable to assess the stress accurately.

The Perceived Stress Scale (PSS), designed by Cohen, Kamarck, and Mermelstein (1983), is one of the most used psychometric instruments to measure stress as judged by the respondents themselves. Several versions of PSS are available including a short 4 items scale, 10 items scale and 14 items scale. The 10 and 14 items scales are commonly used in studies. The PSS uses the five-point Likert scale based on the frequency of the stressful event experienced by the respondent (0 = never, 1 = almost never, 2 = sometimes, 3 = fairly often, 4 = very often). The higher the score of the PSS, the higher the stress perceived by the partcipant. The established reliability of the original PSS is 0.85 [6].

Since PSS was used widely and universally, the original English version of the PSS had been translated into many other languages including Chinese [7], Japanese [8], Spanish-Chilean [9], Hungarian [10], and European Spanish [11,12]. However, no published study based on the Malay-translated versions of the PSS was reported. Since Malay language is the official language in Malaysia, there is an urgent need for a valid and reliable Malay version of PSS.

The current study aims to validate and determine the reliability of the Malay-translated versions of the PSS used among female prisoners. The results of the current study will enable a valid and reliable Malay-translated version of the PSS to be applied for such studies among prison population in Malaysia.

Method

Study design and participants

A cross-sectional study was designed for the current study. The sampling sites were prisons in Peninsular Malaysia having female inmates. Two of the prisons were the sampling frame. Convenient sampling method was employed based on availability of the participants. The participants could communicate, read and write in Malay indepently.

Calculation of the sample size was done separately for factor analysis and reliability testing. The sample size for factor analysis was calculated based on Gorsuch's (1983) suggestion [13]. The total number of items in the instrument is multiplied by 5 and the resulting number gives the required sample for the study [13]. For reliability testing, Cronbach's alpha formula was used to calculate the sample size. The higher value of the two calculations was taken as the final sample size. A 20 percent dropout was included in each estimation. For the current study, the required sample size was 90. For test-retest reliability testing, the calculated sample size was 40.

Translation process

Prior to the validation process, the authors translated the original version of the PSS into Malay. The translation script was read through and checked thoroughly to ensure proper use of word and sentence. This was followed by back-translation where the Malay version was translated back into English by an expert in the field. The expert was ensured that he had no prior knowledge of original English version. Comparison was then made between the English-translated version and the original English version. Finally, a language expert checked through the final Malay version for any grammatical or language error.

Data collection

The data was collected done at prisons situated in the Peninsular Malaysia. The selected participants were informed of the purpose of the current study and all relevant information was communicated. All doubts were clarified and the participants were assured that they could withdraw from the study at any time during the data collection process. Upon their agreement to participate, a respondent information sheet was given and a consent form was signed prior to data collection.

Each participant was given a set of Malay-translated version of the PSS. The average time taken to complete the instrument was three minutes. After completion, it was returned to the researcher. One week later, the same instrument was retested among some selected participants at the same prisons.

Analysis

The data was analysed using SPSS version 19.0. The demographic information was summarized using descriptive statistics. A confirmatory factor analysis was performed to assess the construct validity of the Malay-translated version of the PSS,. The factor structure of the translated instrument was assessed using principal component analysis with varimax rotation, as suggested by previous studies [8]. Prior to the factor analysis, preliminary analysis for factor analysis was examined to evaluate the adequacy of the translated instrument [14]. For the preliminary analysis, the value of the Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy, individual Measure of Sampling Adequacy (MSA) and the Bartlett's test of sphericity were observed. The acceptable limit of the KMO value is .50 [15], whereas the individual MSA is expected to exceed .50 [16]. Items with individual MSA below .50 were excluded from the analysis since they affect the overall value of the KMO [16]. However, the exclusion depends on the value of KMO. Lastly, the Bartlett's test of sphericity is expected to be significant since it indicates the appropriateness of factor analysis for the translated instrument [16].

The assessment of the factor structure was then conducted. Using the SPSS application to conduct the confirmatory factor analysis, the number of factor was fixed as two based on previous studies [1, 8]. The number of factors presented the subscale or content domain of the instrument and each factor explained a certain percentage of variance. Items highly loaded into each factor were examined during assessment of the factor structure (factor loading). Finally, comparison was made to previous studies. To assess reliability, the internal consistency reliability of the translated version was measured using the Chronbach's alpha coefficient (α). For test-retest reliability, the Pearson's correlation coefficient for the total score and individual items was calculated.

Results

Demographic information

The age of the participants ranged from 17 to 53 years old. The mean age was 28.81 years. As shown in Table 1, majority (87.8%) of the participants were Malay. Most of them were married (52.2%), had the secondary education as their highest education (80.0%), and were frequently changing jobs (42.2%). In childhood, most of the participants lived with biological parents (80.0%) and had 4-5 siblings (35.6%).

Table 1: Summary of participants' demographic information (n= 90)

Information	*N*	*%*
Ethnicity		
Malay	79	87.8
Chinese	6	6.7
Indian	5	5.6
Marital status		
Single	22	24.4
Married	47	52.2
Divorcee	16	17.8
Widow	5	5.6
Highest education		
Never been to school	3	3.3
Primary	9	10.0
Secondary	72	80.0
Tertiary	6	6.7
Employment prior to incarceration		
Permanent job	34	37.8
Always changing jobs	39	42.2
Unemployed	18	20.0
As a child, lived with:		
Both parents	72	80.0
Either parent and a stepfather/stepmother	8	8.9
Grandparents	7	7.8
Relatives	2	2.2
Foster family	1	1.1
Number of siblings		
Single child	4	4.4
1 – 3	25	27.8
4 – 5	32	35.6
More than 7	29	32.2

Factor analysis

For the preliminary analysis, the KMO Measure of Sampling Adequacy value was equal to .70. The individual MSA ranged from .63 to .78. Since all items had individual MSA more than .50, none was excluded from the analysis. The Bartlett's test of sphericity was also significant ($p < .001$). Overall, the preliminary analysis was satisfactory.

Based on the original version and previous studies [1, 8], two factors were extracted from the Malay-translated version of the PSS. Factor loading of the translated instrument was similar to the original version (as shown in Table 2). Items in Factor 1 were item number 1, 2, 3, 6, 9, and 10. The items represent negative perceptions, which explained 27.37% of variance. Items in Factor 2 were item number 4, 5, 7, and 8. Factor 2 represents positive perceptions and explained 22.64% of variance. Both factors explained half (50.01%) of the variability in the Malay-translated version of the PSS.

Table 2: Factor loadings for the Malay-translated version of the PSS

Items No	Factor loadings	
	Factor 1	Factor 2
1	.589	
2	.628	
3	.649	
4		.767
5		.715
6	.662	
7		.532
8		.798
9	.802	
10	.612	

Reliability testing

The Malay-translated version of the PSS produced the Chronbach's alpha of .64. For individual factor, the Chronbach's alpha for Factor 1 was .74 and for Factor 2 was .68. Pearson's correlation coefficient, which represents the test-retest reliability, for the total score was satisfactory (R=.72). As shown in Table 3, individual items showed that most of the items had the Pearson's R lower than .50 with the lowest being item number 9 (R=.27) and the highest was item number 5 (R=.63).

Table 3: Pearson's coefficient (R) for individual item and total score for the Malay-translated version of the PSS

Items	Pearson's R
Item 1	.31
Item 2	.41
Item 3	.42
Item 4	.51
Item 5	.63
Item 6	.38
Item 7	.43
Item 8	.49
Item 9	.27
Item 10	.49
Total	.72

Discussion

As the preliminary analysis confirmed that factor analysis was appropriate for the Malay-translated version of the PSS, two factors were extracted from the instrument. The item loadings were found matched to the original version and a previous study [1, 8]. As suggested by the original version, the two factors represent negative and positive perceptions of stress among the participants [1, 8]. No problem was found with the item loading since all the items highly fit into their respective factors. The finding showed that the Malay-translated version of the PSS was valid for use.

The published internal consistency of the original version of the PSS was very high (α = .85), demonstrating the high reliability of the original version. Note that reliability refers to the consistency of an instrument to measure a construct such as psychological construct when it is given to the same person at a separate time or given to a different person under a similar condition [15]. The Chronbach's alpha is suggested to be within .70 and .80 for the instrument to be considered as reliable [15]. However, in the case of a psychological construct, variety of the constructs being measured may affect the Chronbach's alpha value to go below .70 [17]. Number of items in an instrument also affects the value Chronbach's alpha heavily [18]. In the current study, the total Chronbach's alpha of the

Malay-translated version of the PSS was lower than .70 (α = .64). This might be explained by factors such as number of items and the psychological construct. Nevertheless, it exceeded the acceptable value (α > .50). On the other hand, the individual Chronbach's alpha for both factors were higher than the total Chronbach's alpha, with factor 1 had the Chronbach's alpha above .70.

The Malay-translated version of the PSS had high total Pearson's correlation coefficient (R=.72). The test-retest reliability evaluates the consistency between two measurements when an instrument is given to the same person twice [19]. Several factors such as the time interval between test and retest [20], and the effect of memory [21] may affect the test-retest reliability. The individual Pearson's correlation coefficient demonstrated inconsistency between test and retest in most items (R < .05). Item number 9 which had the lowest correlation coefficient (R=.27) required the participants to recall past experiences: *In the last month, how often have you been angered because of things that were outside of your control?* The inconsistency in response to this item might be explained by the memory effect [21]. Nevertheless, the total correlation coefficient was high and considered reliable.

As mentioned earlier, the PSS had been translated in many other languages. This included the 14-items version of the PSS. Several published studies on the translated versions were found [7-12]. The 10-items version of the PSS had been translated and validated in Japanese [8]. The Japanese version of the PSS was termed as PSS-J and it was compared to the original version in the study. The participants were pharmacy and nursing students who were recruited in London and Tokyo to respond to respective versions of the PSS. In the study, exploratory factor analysis was conducted, which revealed two factors structure explaining 42.6% of variance. The internal consistency of the PSS-J was .74. No test-retest was conducted in the study [8].

The 10-items version of the PSS was also translated in Hungarian with comparison to the 4-items and 14-items versions [10]. Participants from a stress-management program were recruited in the study and a test-retest was conducted after five days. The Chronbach's alpha of the Hungarian 10-items version of the PSS was .85 with the Pearson's correlation R equal to .99. In the study, all three versions of the PSS were found closely correlated to each version [10]. In European Spanish version, the internal consistency of the 10-items version was equal to .81 [11]. Groups of people who were possibly highly stressed such as HIV patients were selected as the participants. Both the 10-items and 14-items versions were applied in the study. Test-retest within two weeks interval produced the Pearson's correlation coefficient of .77 [11]. Several translations of the 14-items version were also reviewed. In Chinese, the Chronbach's alpha of the 14-items version was .81 [7]. In another European Spanish version, the 14-items PSS produced the internal consistency of .83 [12]. Confirmatory factor analysis was conducted in the study, revealing two factors structure of the 14-items version. Nonetheless, none of the previous studies involved prisoners.

The limitation of the current study is associated with the study population, which involved only female inmates. No control group or free-living participants were taken. It is also worth noting that the prisoners experienced more stressful situation than free-living people. The validation and reliability testing thus might or might not be affected. Therefore, the validity and reliability of the current Malay-translated version of the PSS should be more specific for female inmate's population rather than Malaysian in general. Based on this limitation and with reference to the Malay-translated version of the PSS, a local psychometric instrument could be designed to measure stress in the future.

Conclusion

The confirmatory factor analysis and reliability testing of the Malay-translated version of the PSS were found to be satisfactory. The Malay version of the PSS conformed to the original version and to previous studies, indicating the validity and reliability of the Malay-translated version of the instrument to measure stress among the Malaysian population, especially female prisoners in future study. Further study using different population samples is highly suggested with reference to the findings and limitation in the current validation work.

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