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Dr. Ranjeet K Singh
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Traced Forgery – A Case Study

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Abstract:

In today scenario signature has great importance, as every individual signs his or her name once daily or several times in a day. Due to frequent use of the signature, the question often arises that whether the signature is genuine or not. Signature Forgery simply means altering any signature with the intent to defraud which could be executed by numerous methods. One of the method which is discussed in this case is traced forgery, which means reproducing the exact copy of the genuine signature. Traced forgery is executed by using carbon paper, indented tracing, tracing paper, transmitted light or scanned image. Forensic Document Examiner examines the every minute details of the traced signatures and conclude whether the questioned signature shows the resemblance and similar mathematical measurements in respect of model signature or not. Here in this paper, a case has been taken in which three disputed signatures is examined for giving probable conclusion. First the inter-se comparison of questioned is done followed by the comparison of signatures with admitted for finding the origin of the questioned signature. The result in this particular case, revealed that the three disputed signature exactly superimpose with slight adjustment.

Keywords: *Handwritten Signatures, Forgery, Tracing*

Authors:

1. *Sherlock Institute of Forensic Science, INDIA*

Introduction

Handwritten signature is being used on regular basis, because of its frequent use it is always questioned. As many a time people tries to imitate the signature of an individual for their personal benefit or for harming an individual or an organization. Therefore there is need for verifying the signature to know who actually signed the document. But before understanding how to verify that the particular signature is genuine or not, first the concept of signature must be clear. Signature is generally defined as a name or a mark that a person puts on a document to authorize that the document has been crosschecked by him/her (Mathyer, 1961). The signature is classified into Formal signature, Informal Signature and Careless scribbled signature which the writer execute as per need or requirement (Saferstein, 1982). But currently the signatures are frequently the subject of forgery (Jackson and Jackson, 2011). Forgery means to dishonestly making, altering, or writing one's signature, which is usually tried by offenders in most of circumstances with the intention of making money from the acquitted parties (Lerner and Lerner, 2006). There are number of forger who prepare the forged documents either by trickery or by using different method of forgery such as tracing, simulation, transplanting, and computer generated document. The most commonly used method is tracing, a mechanical act in which forger imitates exact copy of the genuine signature. For tracing a signature the forger must have a model signature to copy and if the forger successfully execute the tracing then the forged signature will show exact mathematical measurement, which can be verified by using superimposition technique (Misra *et al.*). In current scenario, according to the forensic document experts, a traced forgery is the crudest forgery. Below mentioned are the different of tracing forgery (Sharma, 2003):

1. Carbon Copy Tracing – In this method, carbon paper is positioned between the genuine document and targeted document, then the signature on the genuine document is outlined with the help of a dry pen, stylus or any other pointed device with pressure. Carbon copy is completed with ink and pen.

2. Indented Tracing – In this method, the genuine document containing the model signature is positioned over the targeted document, then the signature is outlined with a sharp and hard point with pressure. Signature on the targeted document is covered with ink and pen.

3. Tracing Paper – In this method, tracing paper is positioned over the genuine document and a hard pointed pencil is used for obtaining the intended impression. Indented impression is completed by ink

4. Transmitted Image – In this method, genuine document is positioned on the top of the glass table and a light is passed from below of the table. This will help the forger in outlining signature from the genuine document on the targeted document which is inked later on.

5. Scanned Image – In this method, scanner is used to copy the genuine signatures and store the digital image of signature in computer and used in future as per required.

Hence, after concluding that the particular signature is forged the next question comes in the mind of forensic document examiner is to identify the forger. In the field of questioned document, identification of signature and handwriting is necessary (Levinson, 2001). The identifying feature that should be taken into consideration while dealing with traced forgery cases are as follows abrupt changes in the direction of the line, lack of fluency, more line tremors, pen lifts, blobs of ink, retracing, and poorer duplication of the line quality than free-hand forgeries. In carbon paper tracing method, residues of carbon along the inked lines under microscope, indentation which can be visible under Electrostatic Detection Apparatus (ESDA) (Hilton, 1939; Koppenhaver, 2002; White, 2004). Here in this paper, we had discussed a case on traced forgery with an aim to identify the method used for tracing and to know the origination of the questioned signature.

Material and Method

This section of the paper describes the material and method used for conducting the examination of the unique case received at Sherlock Institute of Forensic Science, India. The material required for conducting the examination are photocopied signature sample of the case, hand lens, scales, pencil, transparent sheet, camera as shown in figure 1. For studying this particular case, the three disputed signatures were marked as Q-1, Q-2 and Q-3 which was obtained from Standard Instructions, Most Important Document (MID) and Addendum respectively and admitted signature marked as A-1, obtained from Bank Attested Signature.

Figure 1: Material Used

Result

In this case, the questioned signature were marked as Q-1, Q-2 and Q-3 and admitted signature as A-1 were written as "r, i, t, u, a, g, a, r, w, a, l". On inter-se comparison of questioned signatures marked as Q-1 to Q-3, mathematical similarities was clearly observed which indicated that forgery is executed either by paper tracing or glass tracing. Further when compared the features of signature via transparent sheet, it was observed that the questioned signature exactly superimpose with other questioned signature with slight adjustments as the forger also know that exact tracing will put them in trouble. The overlapping of the letters and strokes with each other executed with slight variations, which indicates the sign of traced forgery through transmitted light either by Glass tracing or paper tracing. During reconstruction process, it was found that it was a case of mechanical transplantation of one genuine signature on different targeted documents.

On inter-se comparison, the questioned signature marked Q-1 to Q-3 shows tremors, unnatural pen lifts, line quality defects. While during examination of every single letter formation, it was observed that the letter "R" of "Ritu" in all three questioned signatures were found exactly superimposing in respect of stem, counter and leg of "R". Leg of "R" is the initial stroke of "i", the crossbar with tapered end and downward extension of the stem of the succeeding letter "t" and garland type formation in letter "u" superimpose in all three questioned document with very slight adjustment as shown in figure 2. In word "Agarwal", the hook formation in letter "A" and connection of crossbar of letter "A" with the initial stroke of letter "g" shows similar pictorial representation. Connection between letters "a, r, w, a, l" are consistent and superimpose in all three questioned signature with very slight adjustment as shown in figure 3.

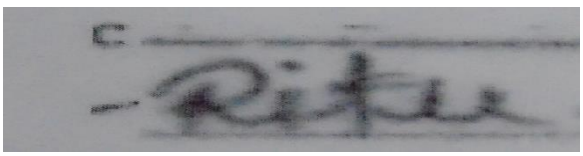

Figure 2: Superimposing of Questioned Signatures in word "Ritu"

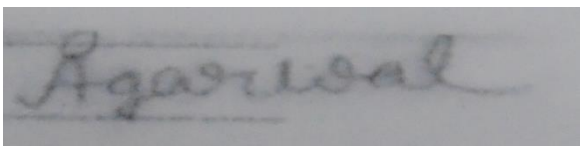

Figure 3: Superimposing of Questioned Signatures in word "Ritu"

Further when the questioned signatures was compared with admitted signature for finding the origin of the signature, it was observed that the questioned signatures was superimposing with admitted signature with very slight adjustment as shown in figure 4. The similar mathematical measurement and overlapping of the questioned signature with admitted signature proves that the disputed signature must have been traced from the admitted signature either via glass tracing or paper tracing.

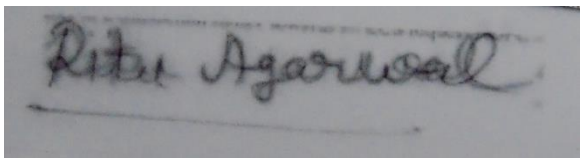

Figure 3: Superimposing of Questioned Signature with Admitted Signature in word "Ritu Agarwal"

Discussion

In present study, a case has been taken in which the three questioned signatures were examined that whether they exactly superimpose or not during inter-se comparison followed by the comparison with admitted for finding the origination of the questioned signature. There are already a case study given by Misra *et al.*, in which he had studied a similar type of case. In which they concluded that 95% superimposition of genuine signature with forged signature will results into Traced Forgery on inter-se comparison. Here in this case study, it was clearly observed that the questioned signature shows similar mathematical measurement and superimposition during inter-se comparison. Further when compared with admitted signature, it was concluded that the questioned signatures must have been traced from the admitted only either via glass tracing or paper tracing.

Conclusion

The basic principle of handwriting states that "No two writing can be alike of the same writer also" and the same principle applies in this particular case also. In this case, the questioned signatures shows similar mathematical measurement and exact superimposition on inter-se comparison and on comparison with admitted, concluded after the examining it with the help of transparent sheet. The observation extruded out from this case, proves that whatever method a forger will use to imitate the signature, it can never be executed as the genuine one of the genuine writer.

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A review of Credit card Fraud Detection techniques in e-commerce

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Abstract:

With the rise and light growth in e – commerce, the use of credit card for online transactions is also increasing dramatically. Due to this there is a great amount of increase in credit card frauds for which there is a requirement of various detection techniques for determining the fraudulent transactions. Frauds can either be offline or online for regular purchases, credit card is used as a mode of payment. Fraud is considered as the most ethical issue in credit card frauds and it is a million dollar business which is rising every year. Recent advances in techniques based on Data mining, Algorithm system (Genetic algorithm, Artificial Algorithm), Machine learning, Hidden Marokov model are the modern techniques that are introduced for detecting credit card fraudulent transactions. This paper reviews all the fraud detection techniques which have some advantages and disadvantages as well. As according to the study Hidden Markov model and Data mining techniques are considered as the best suitable techniques and may be considered over other techniques successfully.

Key Words: *Credit card, fraud detection, Data mining, Hidden Markov model, and e – commerce,*

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Introduction

Fraud generally refers to obtaining money and goods/services by wrongful or criminal deception or in illegal way which intends to result in personal or financial gain. Fraud deals with criminal events that needs identification which becomes difficult. Due to the development of technology and the increase of internet usage, credit card frauds or can say online frauds are increasing day by day. This wide ranging term "credit card fraud" used for theft or any fraud committed or any transaction that is done as to gain a fraudulent source of funds in a transaction. Credit card fraud is defined as when an individual uses another person's credit card information for his/her personal use without the consent of the owner. These credit card frauds need immediate detection which should be active. In the cases of web technologies the research is necessary as it supports E-commerce in managing and building these applications. This business of E – commerce makes it possible to shop anything anytime as it have no time and geographical restrictions.

Detection of these frauds is difficult task if using normal process of detection, so the use of models i.e., fraud detection models is considered as of more importance in cases of academic or business organizations. E- Commerce are of many kinds – B2B (Business to Business), B2C (Business to Consumer), C2C (Consumer to Consumer) are the three popular application forms of E-commerce. As we have discussed number of models/ systems/ configuration/ process and some preventive measures that are used to avoid credit card fraud which reduces financial risks. These include some common cases such as acquiring or property trading which includes personal and intangible property such as stocks, bonds and copyrights. It is becoming essential to combat these type of fraudulent transactions for which various techniques were applied such as Hidden Markov Model, Artificial Intelligence, Sequence Alignment, Data Mining Techniques, Multiple cryptographic Algorithms and Genetic programming techniques (Rana and Baria, 2015; Meshram and Yenganti).

Types of Frauds

Credit card frauds are mainly divided into two classes:

- *Offline frauds* – are committed by using credit cards that are stolen at place.
- *Online frauds* – are committed via internet, shopping, phone, and web or in case of absence of credit card (Rana and Baria, 2015).

The definition of problems related to credit card frauds

An e-commerce business or system provides suitability for online transactions to shop anything to be a universal business. These frauds can be possible in both ways online as well as offline.

1. While online transactions such as during shopping, fraudster only uses the card information or just want to harass Merchant or to the banks by doing frauds:
2. In case if fraudster don't want to buy anything from the shop but still he/she provides wrong information and payment was done via cash on delivery to harm the merchant.
3. If the credit card of the user was lost or stolen then the fraudster can use the information of card like Credit card number, CVV number to make payment without the consent of cardholder (Rana and Baria, 2015).

Proposed Systems for credit card fraud detection

Various credit card fraud techniques were developed by Researchers.

Data Mining Technique

The technique of Data Mining uses complex data analysis tools that discover the unknown, valid patterns and association among large data sets. The analysis tools include statistical systems, mathematical algorithms and machine learning methods like Neural Networks. This method comprises of collection and maintenance of data which represented in the form of textual, quantitative or in multimedia forms that also involves examination, estimation and prediction. These application uses range of factors to observe data which includes association, classification, sequencing or path analysis. Banks contain large databases which have suitable information on fraudulent acts related to the banking Industry. The important business information was extracted from the stored data and these stored data patterns as clusters naturally inputs some data. The foremost principle while detection is matching the abnormal pattern with the normal pattern. Huge amount of e-commerce industries depends on credit card purchases which is more important for business and organizations (Chaudhary and Mallick, 2012).

Algorithm Technique

Artificial Intelligence (AI), algorithm was used or introduced for the purpose of detecting credit card/ATM cards, cheque books type of fraudulent documents.

Cryptographic Algorithm - According to Meshram and Yenganti, Cryptographic techniques were used for preventing fraud by using tools which asks for secret, questions. The multiple layers of security were enhanced using security questions which wraps the pin number in previous stages using Cryptographic algorithms. With the use of this method fraud can also be prevented (Meshram and Yenganti, 2013).

Genetic Algorithm is another method of detecting credit card frauds as this make use of the existing transactions of credit cards which involves multiple standards for detection. These standards include frequency and location of card usage and balance on credit card book. The filter and priority settings are also responsible for detecting frauds (Alekhya and Basha, 2013).(Bentley et al. 2000) suggested one algorithm that is classified on the basis of credit card transactions into suspicious and non-suspicious classes in genetic programming. Due to the employment of different type of rules were tested in different field in which the best rule is of the high predictability. For the determination of suspect behavior an algorithm was developed in the model cost and rate were evaluated whereas in other cases use of different evaluation techniques are done on the basis of prediction rate and the error rate (Chan et al 1999).

Hidden Markov Model

Hidden Markov Model was used as a detection technique which is used to sequence the model of operation during the credit card transaction which is done by using Baum – Welch algorithm. The transaction was considered as fraudulent if the incoming transaction of credit card was not approved by trained Hidden Markov Model (HMM) with sufficiently large enough probability. Three behaviors of card user were taken into response to detect fraud.

1. Low spending behavior (spending low amount)
2. Medium spending behavior (spending medium amount)
3. High spending behavior (spending high amount)

The spending behaviors are different for different card holder which includes low spending behavior (Ingole and Thool, 2013).The need of training data and test data is always required to detect fraud which uses data mining algorithm K- means internally. Due to this K – means algorithm all credit card information is taken, input data is the number of clusters and these are used in HMM technique. The amount in this technique can be either low, high or medium (Alekhya and Basha, 2013).Abhinav Sruvastava et al proposed a recent research on HMM technique which is a double surrounded random process of probability distribution or pattern with two levels of hierarchy. It can be considered as model which may be more complex process when compared to the traditional Markov model. Many problems exist while using HMM technique such as it does not support many systems or not compatible with some systems.

Outlier Detection

This outlier detection is used for fraud detection system which is generally an observation that diverge from the path of another observations that arises from suspicion. Learning approach that was not supervised is employed by this model which results in a new representation of the observed data. The normal behavior was represented by this method which involves modelling of baseline distribution whereas on other sides i.e. supervised methods in which the models are exercised to differentiate between fraudulent and non-fraudulent transactions through which new observations are assigned. In case of unsupervised methods there is no need to have some prior knowledge about fraudulent and non-fraudulent documents (Chaudhary, Yadav and Mallick, 2012).The outlier detection technique is used where large scale data is involved and this technique works efficiently for applications where computational limitations are present (Pawar et al, 2014). (C. Aggarwal and P. Yu) A method of outlier detection were used for high dimensional data space and the behavior of data of projectiles is required to find the outliers.

Bayesian and Neural network

BNN is a fraud detection technique that is automatic based on machine learning approaches. This techniques does not need to be reprogrammed and this considered as a great advantage of neural network. Neural network processing speed is high in comparison to Bayesian and Neural Network and a high processing time is required for huge neural networks. This fraud detection technique i.e., Bayesian and Neural networks provides good accuracy. The number of disadvantages in case of Neural Network systems such as structure confirmation is difficult, needs excessive training, training efficiency and so on. (Dorronsoro et al 1997) developed a system that is based on the neural classifier system that has limitations that the clustering of data is must done by any type of account. Leonard 1995 explained one disadvantage that is mainly the time constraint while using a rule based expert system in Neural Networks. (Brause et al, 1999) allows the use of neural network technologies which have been used in fraud detection

techniques whereas (Ezawa and Norton, 1996) explained one more technique that is Bayesian Network is also used as an alternative to other methods and applied in telecommunication industries.

Hybridization technique

This is basically sequence alignment of two stages and two analyzers, profile and Deviation analyzer. With the help of Profile analyzer the sequence was matched with the cardholder database whereas in Deviation analyzer, the past history of fraud database was compared with incoming unusual data as the unusual data is passed on to the deviation analyzer. The system was alarmed in case of fraud. The major limitation with this system that it does not help in the detection of cloning cards (Rana and Baria, 2015).Rilly have proposed the technique of BLAST-SSAHA Hybridization technique through which the detection improves by combining both individualities as well as misuse detection.

Literature review

Researchers developed many fraud detection techniques through which the occurrence of frauds are decreasing.

Ghosh and Reilly 1994 advanced neural network for the detection of frauds which should provide a large sample of credit card transaction that is labeled. The cases related to these type are stolen of credit cards or lost cards, counterfeiting, application frauds, non-received issue (NRI) fraud and fraud through mail order.

Alekerovet et al. 1997 recommended in their presentation CARDWATCH that a neural network system based on the data mining process. This is generally a trial product based on data mining process that is developed for credit card fraud techniques and a basic requirement of this system is one network per customer.

Rumelhart 1986 states that organization of nodes into layers and after layering, the layers of neurons were attached with interconnections of modified weight. The new environment of its own behavior matched with the present environment to the new possible situations.

Quinlan, 1993 developed a decision tree method that is a learning system that deals with uniform data and has also developed ID3 method which is more advantageous as it has high flexibility due to which the distribution of data may be done without any assumption and the other one is the robustness which is the main reason for its good utilization.

Fan et al 1999, recommended the use of data mining technique i.e., distributed data mining in fraudulent transactions or detection of credit card frauds. This is considered as the well-organized way of arranging highly distributed databases and Boosting algorithm was used to detect system. Ada Cost is the other name given to Boosting Algorithm which uses classifiers in large amount and more resources of computational devices during detection.

Lane 1999 recommended the use of HMM model to human behavior as after the correct modelling of human behavior, the deviation that is detected must be observed. The behavior of attacker was not found to be similar to authentic user.

Stolfo et al.1999 suggests a Meta learning techniques that can be used for credit card fraud detection systems. This technique of Meta learning combines and divide or integrate the numbers separately to build models. For the detection of fraud and intrusion, the same group worked on it as they use Java agents for this process. The number of important metrics such as the distribution and accuracy of true positive-False positive (TP-FP).To learn models of fraudulent transactions and to acquire findings of high fraud along with low forged alarm this process is considered as suitable for fraud detection.

Syeda et al 2002 proposed a technique that with the use of equivalent large or rough neural network the speed can be enhanced and also suggested the Knowledge discovery process (KDP) that helps in acquire the speed up to 10 processors only but in case of introduction of more number of processors leads to problem of imbalance. For increasing the data mining speed and the discovery process of knowledge use of parallel granular neural networks (PGNNs) is suggested. Due to the complex nature of Hidden Markov Model, the series of time are not scalable or measurable to large size data sets.

According to the suggestion of Cho and Park 2003, HMM based intrusion technique were developed that helps in enhancing the time of modelling and performance. This was improved by the help of privilege transition flows which is based on the knowledge of the domain attacks.

Hoang et al 2003 proposed a new method by using HMM to process sequencing of system calls for anatomical detection.

Joshi and Phoba 2005 examined the abilities of Hidden Markov Model in anatomy detection which classifies the traffic network in the form of attack or normal using HMM.

Abhinav Srivastava et al 2008 suggested that the credit card fraud technique that is Hidden Markov Model (HMM) shows 80% accuracy for acquiring large variations in the input data.

Amalan Kundu et al 2009 suggested that a model of BLAST-SSAHA based on hybridization technique for the online credit card fraud detection. This system improves the detection technique which involves the combination of individualities and misuse techniques of detection.

Alekhya and Basha, 2013 discussed about the E-commerce applications in credit card frauds. The genetic algorithm, a technique applied for the detection of frauds are implemented. In this Java platform was used by a prototypic application and genetic algorithm technique is basically used to detect frauds related to credit cards. This paper preferred Genetic algorithm over other techniques and it is proved by experimental results.

Ingole and Thool, 2013 proposed that HMM technique was used in detection of credit card fraud by sequence modelling of credit card transactions involves the use of the clusters created by clustering algorithm. An HMM technique is used with Baum-Weich algorithm which detects that whether the transaction i.e., incoming is fraudulent or not. The performance of systems was calculated by using metrics and the observed accuracy comes out to be 75%.

Meshram and Yengati 2013 performed experiment on cryptographic algorithm in which a system was proposed that the identity of exact user can be found not only by using security pin number but by embedding secret questions. By selecting the appropriate path for the transferring of any file from source to destination, file can be saved in its own destination and then the transferring process takes place that is fully secured. ‘

Shabbir and Kannadasan 2013 proposed the method of mining technique proves that fraudulent transactions can be deduced and number of false alerts can also be reduced. The genetic algorithm method when applied into the credit card fraud detection systems in bank through which the chances of fraud transactions can be predicted earlier than the credit card transactions.Anti-fraud strategies were applied to avoid frauds related to credit cards.

I. Discussion and Conclusion

(Ingole and Thool, 2013) proved in his research paper that the performance was calculated on the basis of metrics by using HMM technique is 75% whereas according to Abhinav Srivastava et al have proposed 80% accuracy in Hidden Markov Model in input data of large variation . The swindle detection based on outlier detection can detect credit card frauds better than data clustering technique i.e., Hidden Markov Model .Genetic algorithm is considered as a novel technique related to domain of application that when the algorithm was applied onto the bank credit card fraud detections, the probability of fraud transactions may be estimated after the credit card transactions. And through these techniques new strategies or anti-fraud strategies were adopted to prevent from bank frauds and reduce risks (Shabbir and Kannadasan, 2013) but According to Philipi and Sherly,2012 Algorithm techniques has some disadvantages which required specialized and supervised training to sufficiently optimize parameters. With the increase of fraud detection techniques, there is an increase in fraud detection systems.(Pawar et al, 2014)The algorithm techniques has some disadvantages as this requires extensive supervised training, testing and all these requires involvement of human to prepare for training and test cases to sufficiently optimize parameters. Outlier detection techniques were also used which requires large scale data and are used with those applications where memory and computational limitations are present. So (Chaudhery,Yadav and Mallick, 2012) explained in his paper, 13 different classification systems were determined and all these models/system describes the advantages of Data mining technique in Artificial Neural Networks (ANN) for the detection of fraud transactions. As the distribution of training data sets becomes more partial, with this there is a decrease in the performance of all models which directly makes difficult the capturing of fraudulent transactions. (Chaudhey, Yadav and Mallick, 2012) that they considered practice research unusual in case of statistical methods and Neural Network system has advantages and disadvantages as well. The number of disadvantages in case of Neural Network systems such as structure confirmation is difficult, needs excessive training, training efficiency and so on. (Philipi and Sherly, 2012) described in their paper that the algorithm method is considered as more effective in real world as arrangement of data sets is extremely efficient which involves the study of linear and nonlinear relationships that comes directly from modelled data that is in a form of linear fashion. Rama and Baria, 2015 stated in their paper that good accuracy obtained from Bayesian neural networks but required high processing speed and data training to operate it. According to Ghosh and Reilly, 1994, the technique of data mining requires long training time. The proposed limitation for a neural network system based on data mining is that it require one network per customer (Aleskerov et al).Baysian method has advantages of high accuracy, processing speed is good, false alarm reduction, detection rate is improved

and a good application in e- commerce but one disadvantage is that it is highly expensive. According to Rana and Baria, hybridization technique is considered as inexpensive and have high accuracy, HMM and Bayesian neural network technique is quite expensive with medium accuracy. (Rana and Baria, 2015). After reviewing every details about detection technique, we may conclude that Hidden Markov Model, Data mining systems and Genetic Algorithm are the best ways to detect fraudulent transactions.

In this review paper, study is related to the detection of credit card fraud and the techniques related to E-commerce applications. Various detection systems were explored to solve the problem of frauds as the information about several detection methods can help to improve and protecting the applications of E-commerce. Every fraud detection techniques has its own advantages and disadvantages or strengths and weaknesses. In E-commerce, rising of an accurate and credit card fraud technique that is resourceful is necessary. Finally the implementation of some methods such as Genetic algorithm, Hidden Markov Model, Neural Network, Data mining techniques were done. While doing the comparison of performance for predicting the accuracy, this survey or review results that the application used to detect fraud is useful and is used in real world systems. In this study, we characterized the impact of fraud and the techniques of fraud detection methods as well and how these models helps in capturing the fraudulent transactions.

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Importance of Graphological Studies in Forensic Science: A Review

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Abstract:

Graphology is a scientific method of identification, evaluation and understanding the personality of an individual. Personality mainly comprises the characteristic patterns of thoughts, feelings and behavior which imparts uniqueness to a person. Graphology is not only concerned with the written symbol itself, but also with the symbol's expressive value i.e. it does not depends on the matter in what context the symbol is used or whether it is neat or untidy but rather it focus on what the symbol suggests about the writer. Graphology is found to be useful for the retrieval of information related to the health issues, morality and past experiences to hidden talents, and mental problems. This present study deals with the reliability and validity of this method in the field of forensic science for the sake of dealing with criminal mind.

Key Words: *Graphology, Forensic Science, Handwriting, reliability, validity. Criminal mind.*

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Introduction

Graphology or the handwriting analysis is the field of identifying and understanding the behaviour, personality and characteristics of an individual by analysing the handwriting of a person. As we all know that handwriting analysis is a complex and a very time consuming process. The graphology as a science is not

new to the world it is being in used from the past 400 years. Camillo Baldi is considered as the father of graphology by giving the systematic observation of handwriting in the year 1622. The term "graphology" was coined by Abb Jean-Hippolyte Michon in Paris in 1897 by the combination of two Greek words 'graphein' which means 'to write' and logos which means 'science' (Deshpande, 2016).

Handwriting is such a unique feature of an individual, it doesn't matter that the person with the use of hand, foot or mouth the handwriting will same as well as the unique one. Handwriting is not handwriting it is basically the brain writing as with the response from the brain the person is able to write. A unique pattern is produced by the brain which is as similar with every individual that reveal the personality trait. Handwriting alone can be able to reveal various elements of an individual behaviour or its personality. It is also able to give the brief knowledge about person's intellect, his emotions, fears, aptitude level, skill, their motivation and integrity. (Kedar et al, 2015).

The graphological studies relate with two basic approaches i.e. graphical analysis of the structural type of writing and analysis of the type of symbol or letter. The forgery in any other person's handwriting can be analysed and identified by proper examination of the characteristics like pen pressure, spacing, formation of letters etc. (Dang and Kumar, 2014).

Graphology and handwriting analysis can be classified as the subset of the study of psychology. Graphology is useful in understanding personality traits through the strokes and patterns revealed by individual's handwriting which itself signifies the traits such as emotional and mental instability. Hence these traits are important to be known and can be helpful in dealing with the cases of profiling of any serial killer or any criminal and suspect so as to narrow down the search of an investigation.

The goal of graphology is simply to determine the personality characteristics of a person with the help of the handwriting which reveals the expressive behaviour and its individual style of response (McNeal, 1967).

The graphological studies plays a very important role in the forensic science for dealing with the cases related with psychiatric activity of any criminal or the cases related with any suicide notes, anonymous letter etc. Graphology in forensic is the study of handwriting especially that found in ransom notes, poison pen letters or blackmail demands.The identification of a criminal on the basis of its personality which are also helpful in finding the modus operandi of any serial killer or an insane criminals.

Review of Literature-

McNeal (1967) suggested that during earlier time graphology had been used as a technique for marketing. Hence in his paper he also analysed the data so as to get to know the personality trait of a consumer. He also discussed about the validity and reliability of graphology. He found grapho analysis also helpful in giving the information fast and inexpensively.

Gluhchev (2007) suggested the importance of handwriting analysis in the field of forensic investigation. His goal is to make the handwriting investigation system with the involvement of computer based techniques. The method used i.e. the image enhancement technique and the image segmentation technique are used. Image enhancement technique are helpful in improving the quality of image of any handwriting sample which are found in a bad quality and the image segmentation method is helpful in segmenting the image by eliminating the background and the separation of the rows and separation of words. Hence with the involvement of computer based system the graphological analysis is done.

Coll et.al. (2009) suggested the method which are used for the extraction, analysis and classification of the writing from the psychological approach. This method is used as a tool for the analysis for the HR professionals whom they consider more reliable than the personal interviews so as to hire the on the basis of the writing. Various features were proposed in their research work like the roundness factor and frequency analysis of any word which is different from person to person.

Dazzi and Pedrabissi (2009) examined the validity of the graphological analysis to reveal the personality of an individual. There work of research were based on the two studies in which the first study deals with the handwriting sample of 101 university student by giving the five big questionnaire. Later the two graphologist were requested to examine the sample with the help of 9-point scale. Another study deals with the handwriting text with the autobiographical content for further graphological analysis. Hence in this work two graphologist and two laypersons were involved. Based on the observation they found that, there is no authentic graphological methods to measure the personality of a person.

Bancila (2012) consider the cases of the patients suffering from a diseases like Schizophrenia. The

sampling is done with the patient suffering from any depressive disorder or syndrome or with the patient who are diagnosed with the disorder. Hence proper segment of the mental illness is considered.

Cronje and Roets (2013) carried the graphological studies as the purpose of clinical or diagnostic method. They said graphology as a holistic approach for the analysis and represents the role of graphology as viable in the diagnostic measure and the psychological assessment. With the fact that handwriting is a unique feature as that of fingerprint, hence in his conclusive part they reveal that the person handwriting seems consistent during the entire time period of his life expect some of the intra variation occur in the handwriting. The use of graphology is not error free and not infallible but are surely helpful and considered as the significant tool for psychologist for the understanding of human mind with the help of graphological studies.

Djamal et al. (2013) used the graphology as for the purpose for revealing the overall picture personality of a person for the selection of the employees. Hence here the research work is proposed for the recognition of handwriting with the involvement of identification of style of signature and digit of character using the network that can predict the personality of an individual. The outcome of the research work is done with the help of the Artificial Neural Networks.

Dang and Kumar (2014) analysed the handwriting by keeping the various characteristics of handwriting in mind so as to examining the personality trait of an individual. In their research performance the evaluation and identification of the signature by calculating the mean square error with the use of Back Propagation Neural Network (BPNN). The neural network is used so as to make it more accurate and valid. The human behaviour is analysed on the basis of the signature examination.

Kedar et al. (2015) proposed handwriting analysis as the emerging field for personality trait information. Keeping in mind that graphology is still an established science because of some of the human error and uncertainty in handwriting sample. They also used the technique for automated personality identification through handwriting i.e. the artificial neural networks technology in which the system is already trained to identify the characteristics traits of handwriting and match it with the relevant personality trait.

Kanchana V et al (2015) used the handwriting analysis for the personal identification of a person. In their work, they use the artificial neural network for the prediction of personality of a person with the help of the extracted features from the handwriting sample. This paper basically focussed with the characteristics of handwriting like baseline, slant and size of letters which are present in individual's handwriting. In this paper, the off-line writer's independent handwriting analysis system is proposed.

Deshpande (2016) use the handwriting analysis for revealing the graphological traits with the involvement of the computerised method named as Back Propagation Neural Network. His research work plays an important role in a way that he used the technique so as to reduce the effort of pen or paper work. Both the use of computational method and the use of software tools as o help and assist the document examiners.

Singh et al. (2016) discussed the case report about a patient of MBBS student who is having a complaint in the change in her handwriting from past long time. Hence the graphological studies is helpful in identifying the reason behind it. Also after the detailed interview with the patient it came to the conclusion that the patient is suffering from the depression of conflict with her 5 year relationship bond. Hence on the basis of viewing different writing sample of her either it's a letter sent to his brother whom she is emotionally attached or letter to her teacher whom she had no emotional attachment reveals the mind-set of a patient. The patient condition were discussed on the basis of the presentation of her high depressive condition revealing the initial complain of her handwriting change. Hence the change in the handwriting converted into the psychiatric symptom of a person.

Discussion and Conclusion

The inclination of graphology and its importance in various investigation of the cases are analysed by reviewing numerous paper on it. McNeal (1967) worked on graphology and initiated it as a new marketing technique for the hiring of employees by analyses of their handwriting samples so as to reveal the personality of the person.

On the basis of the paper of Singh et.al. (2016), the handwriting analysis used a tool for the understanding of an emotional state of a person which can further incriminate during his/her psychiatric judgement. Though it is helpful in revealing the psychiatric condition of a person, so it will surly plays a very significant role while dealing with the cases related with any suicide or criminal insane activity. As with the known fact that the handwriting is a neuromuscular activity the handwriting itself became a very reliable tool for investigating the cases in the forensic world where profiling of a criminals mind, modus operandi of attempting the crime plays a positive role.

Similar type of observation has been taken in the research work of Bancila (2012) where a case study is discussed which shows the inclination of the graho-analysis is helpful in solving the investigation of the case. In this paper it is assisted that handwriting which is useful in giving the additional information for investigators found at the crime scene from the suspect. It is also showed that how handwriting analysis is also turned as a diagnostic feature for the monitoring of patient from psychiatric activity. The criminal or suspect mind is very important and prove to be useful while dealing with the any crime hence the graphological analysis proved to be useful in the forensic investigation of a person so as to narrow down the investigation procedure of any case.

Graphology is an emerging field for the recognition of the personality of an individual. With the help of various analysis techniques one can implement for utilising it for the correct personality information. As we all know that graphology is the applied science but yet not considered accurate due to various human error and the ambiguity in the handwriting samples.

In the present study it has been reviewed and conclusively stated that the graphology is having a great importance in the investigation of various cases related with the handwriting analysis. Automated personality identification through handwriting analysis will proved to be a good and helpful system for personality traits identification. It can find its application in forensic studies by relating it with criminal's mind-set by simply analysing its handwriting..

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Recent Developments in Extraction Methods of Pesticides from Biological Samples: A Review

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Abstract:

Pesticides are the most commonly encountered toxic substances in almost every substrate which includes any environmental sample like water, soil, fruits, vegetables or any biological sample involving viscera, blood, urine and other body fluids. This occurrence of pesticides in the biological samples is because of their tendency of deposition and accumulation in the adipose tissues of the body. The determination of these pesticides from the biological samples (viscera and other body fluids) begins with their successful extraction and isolation from the matrix. The procedure of extraction and isolation depends on the nature of the matrix and also on the selection of the method that utilizes minimum amount of solvent and is capable of providing high yield. Various methods has been developed for this purpose and used to carry out the isolation of these pesticides from the viscera sample, blood and urine and other body fluids. Numerous studies has been conducted to find out the best method to achieve this like liquid liquid extraction, solid phase extraction, and the recent ones including accelerated solvent extraction and others. In this paper a various methods that have been used for the extraction of pesticides from the biological samples is reviewed for determining the best suitable method which has the tendency to provide the results free from the matrix contaminants and have maximum amount of recovery.

Key Words: *biological Evidences, determination of pesticides from biological samples*

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Introduction

Forensic Toxicology is an important discipline of forensic science which deals with the detection and analysis of poisons. Detection of poisons utilizes the application of sequential procedure which involves isolation and extraction of the poisonous substance followed by their separation and analysis using certain sophisticated chromatographic and spectrophotometric techniques. These poisons be it any drug or its metabolite or any pesticide residue because of their tendency of getting deposited into the tissues needs to be isolated from different biological matrixes such as viscera, stomach contents, post mortem blood, serum, urine etc. through various methods (Lanjewar, 2014).

Pesticides are defined as those chemical substances used commonly in agricultural practices to prevent the plants and crops from pests, weeds and other plant diseases. These pesticides being toxic in nature stored in the body of living organisms in fatty tissues and their accumulation increases there over time (Das, 2014). There occurs numerous classes of pesticides based onto the species on which they act. For instance, those which fight against insects are insecticides, similarly those resists fungus are fungicides, and the class of pesticides which prevents the herbs from diseases are known as herbicides and so on. There is another classification which categorize these pesticides on the basis of their chemical nature and function which is organochlorines, organophosphates, carbamates and pyethroids.

These are categorized based on their chemical structure as Organophosphates are the substances that contains phosphate group like Malathion, Dichlorovos, Parathion, Monocrotophos and so on. Similarly Organochlorines are those compounds that contains Chlorine in their structure like DDT, BHC, Eldrin, Endosulfan etc., carbamates are those which are derivatives of Carbamic acid like Carbofuran, Carbryl etc., and Pyrethroids are the ones, which can either be obtained from natural plant source i.e. from *Chrysanthemum cinnarefolium* or these may be synthetic like Cypermethrin, Fenvalerate, Allethrin and so on.

Since, pesticides gets accumulated in the adipose tissues their determination from the matrix poses a serious challenge because of the interference of the large amount of other analytes and substances that can be co-extracted with these and thereby affects the results of the analysis (Tuzimski, 2012).

The protocol used for the preparation of sample is considered to be the most essential step in the detection of these pesticides so as to maintain the sensitivity of the system. Such extraction methods must be of the type that only the relevant or active component or sample molecule is extracted and the other co- extracts and matrix components gets filtered out (Well, 1988).

Nowadays, technology emerged with the development of large number of advanced and modern methods for the extraction of pesticides which are less time consuming and are efficient in providing the maximum % of recovery without the matrix error. Some of these methods includes Soxhlet extraction, Solid Phase Extraction (SPE), and Accelerated Solvent Extraction (ASE), QuEChERS method of extraction and Supercritical Fluid Extraction etc. (Das, 2014).

Liquid Liquid Extraction (LLE) – This is the most widely used technique for the extraction of pesticides from the any biological matrix or sample. The technique utilizes two or more immiscible solvents of different polarity that carries out the selective isolation of the pesticide from any solid or liquid mixture by bringing it in contact with a solvent phase. Process of isolation or separation of any sample (drug or pesticide or other) selectively from the reaction mixture in the form of solution or clear extract or liquid by extraction using another immiscible solvent is termed as solvent extraction (Srivastava and Yadav, 2014).

Steam Distillation – This is another method used for the pesticides extraction through which the steam volatile natural oils, fats, and other co extracts can be removed from the pesticide embedded sample (tissue) (Das, 2014).

Solid Phase Extraction (SPE) – Solid Phase Extraction is the method of extraction that utilizes a solid phase and a liquid phase to isolate different types of analytes from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method (Das, 2014). This method works on the same principle of liquid liquid extraction (LLE) involves partitioning of the solutes between two phases but difference lies in the fact that instead of two immiscible liquid phases as in LLE, SPE method of extraction involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase. Solid Phase Extraction method involves firstly the adsorption of the analytes onto the stationary phase which can be normal polar phase (silica or alumina) or it can be reverse phase i.e. the polar phase is made non polar by replacing the OH group of silica with the incorporation of C18 (carbon octadecylsilyl group). The adsorption causes the retention of the analytes onto the surface of stationary phase which is followed by its desorption or elution

using favorable solvents. The extraction of the sample by this method begins with loading of the sample solution onto the SPE solid phase followed by washing of undesirable components from the matrix and collection of the analyte (Pesticide) into a solution form (Ferenc and Biziuk, 2006).

Supercritical Fluid Extraction- This is another extraction method that involves the sample extraction using supercritical fluid i.e. Carbon dioxide stored at critical temperature 31.3^0C. The process utilizes a thimble in which the sample is placed and the supercritical fluid is pumped through the thimble which leads to the extraction of the soluble compounds of the sample (pesticide) into the collection trap via a restricted nozzle. The fluid is then allowed to vent in to the collection trap which causes the escape of the solvent and this leads to the extraction of the product left in the collection trap. The efficiency of the method depends on the temperature and pressure (Das, 2014).

Accelerated Solvent Extraction (ASE) - Accelerated Solvent Extraction is an automated method for extracting poisonous substance from the biological matrix by the use of organic solvents at an elevated temperature and pressure. The sample is placed in the extraction tube after sectioning into thin pieces which is then placed in the assembly with all parameters set in. This method is advanced as it utilizes minimum amount of solvent and takes less amount of time for the extraction (Lanjewar *et al*, 2014).

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method- QuEChERS is another method developed to overcome the problems associated with the conventional methods of extraction. The process involves the extraction of sample by simply shaking or through vortex mixing using 10 ml of acetonitrile (MeCN). This is followed by the addition of extraction salts (4 g of $MgSO_4$ and 2 g of NaCl) so as to carried out the partitioning of the analytes between the aqueous residue and the solvent. After the aforesaid treatment, it is allowed to centrifuge and the residual water is removed by dispersion Solid Phase Extraction (d- SPE) containing PSA adsorbent and anhydrous MgSO4 salt which are then allowed to mixed with the sample extract for further analysis. This method is beneficial as it requires minimum amount of time than the traditional method of Solid Phase Extraction and also removes residual water and other polar matrix components simultaneously (Deshpande and Srivastava, 2016).

So, pesticides can be extracted from the biological samples (tissues and body fluids) by a large number of methods but the best suitable method is the one which is capable of providing maximum amount of yield and results in highest % of recovery of these pesticides from such samples without the matrix interferences.

Review of Literature

Stalling, et al, (1979) - adopted the method of pesticide extraction using dichloromethane as the solvent from the biological samples but the results obtained are not much efficient because of the not much recovery and wastage of large amount of solvent.

Ballschmiter *et al*, (1981) - worked on the methodology of cold column extraction and doing liquid liquid extraction of the pesticide present in the tissue sample. The extraction is carried out using a 2:1 solvent of acetone and hexane in a gravity fed column that is filled with the pesticide containing dried adipose tissue.

Mes, (1984)- extracted the pesticides from the adipose tissues of visceral samples and other body fluids using conventional liquid liquid extraction by applying cold blending technique for the extracts using a mixture of organic polar solvents like methanol and dichloromethane in 1:1 ratio which gives successful results leads to more than 80% recovery. He also found that other organic solvents including benzene, benzene or acetone mixture does not provide efficient results.

Wells, (1988) - Soxhlet extraction method is used for the isolation of pesticides which involves heating and thereby reduces the polarity of the solvents used, but it leads to a disadvantage i.e. extraction of coextractants (fats) with the desired sample which gets precipitated on cooling and thereby decreases the yield.

Repetto *et al*, (2001) - has developed an analytical method for determination of pesticides from human blood using solid phase technique for the extraction of such samples. The pesticides namely endosulfan, lindane, parathion, ethyl-azinphos, diazinon, malathion, alachlor, tetradifon, fenthion and dicofol are extracted from the blood with the application of a reverse phase i.e. C 18 cartridges during the procedure of isolation. This method of extraction has replaced the conventional method (liquid liquid extraction) i.e. a mixture of hexane and benzene in 1:1 ratio, generally opted for isolation of such samples from body fluids. The extracts obtained are then analyzed with gas chromatography with NPD detector along with the use of two internal standards perthane and triphenylphosphate. The study reveals successful results with recovery range of 71.83 to 97.10%.

Ueyama *et al*, (2006) - carried out the analysis of various pesticides mainly of class organophosphates in the biological samples of human urine. Since organophosphates gets metabolized in the body and

excreted in human urine in the form of Dialkylphosphates (DAPs). These metabolites of organophosphates namely dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), and diethylthiophosphate (DETP) are extracted from the sample of urine by the application of Solid Phase Extraction (SPE) technique along with derivatization using pentafluorobenzylbromide followed by the routine clean up procedure. The extracts obtained when later analyzed by Gas Chromatography- Mass Spectrometry (GC- MS), found that good results were obtained revealing that DMP can be detected approximately upto 0.3 l g/L, and remaining samples DEP, DMTP, and DETP upto 0.1 g/L.

Srivastava and Yadav, (2014) – isolated an organochlorine pesticide Endosulfan from the various biological samples (blood, urine, viscera) using conventional extraction method i.e. liquid liquid extraction and method of steam distillation. The blood is extracted for the pesticide isolation by treating 20ml of blood with 10% solution of sodium tungstate along with the sulphuric acid and shaken for 2 minutes to obtain the acidified clear sample which then extracted with hexane normally using solvent extraction technique and proceeded for further analysis. The same procedure of solvent extraction is applied to the urine sample after doing its refluxing on water bath but the pesticide extraction from viscera is carried out by its acidification using sulphuric acid followed by extraction using steam distillation method.

Lanjewar *et al*, (2014) - performed the pesticide residue analysis of major organophosphorus pesticides namely Malathion, Chlorpyrifos, Monocrotophos, Dimethoate and Dichlorovos, using accelerated solvent extraction method followed by analysis through Gas Chromatography employing NPD as detector. The samples of organophosphorus pesticides are extracted from 20 gm. of viscera mixed with diatomaceous earth material and cut down in small pieces so that a dry mass is obtained for its easy transfer into the extraction cell fitted with a cellulose disk at the bottom. The entire mass is then loaded onto the extraction cell, capped and placed in the tray slots of ASE. The collection vials are then placed at the lower level of the assembly of the method, parameters are set and samples are allowed to extract, dry using sodium sulfate and concentrate for further analysis using GC. Different pesticides are recovered at with different recovery rates differences in limits of detection. Like 0.05ug of Malathion is recovered with 99% recovery, 0.05ug Chlorpyriphos is recovered with 90% recovery, 0.5ug of Monocrotophos is recovered with 90% recovery, 0.05ug of Dimethoate is recovered with 72 % recovery and 0.05ug of Dichlorovos is recovered with 85% recovery when extraction is carried out using Accelerated Solvent Extractor (ASE-200).

Chutke *et al*, (2016) - extracted and isolated fenvalerate, a pesticide of the class pyrethroids by ASE-200 by heating at 70°C and 80°C in three cycles using a mixture of solvents ethyl acetate and acetonitrile (8:2) by spiking the 20gm of viscera sample with 10mg of Fenvalerate and placed it in the extraction cell. The results of the study showed that at 70°C, 86% of the pesticide is recovered which is better in efficiency than the manual extraction procedure and the amount decreases to 62% because of the extraction of the fats and lipid components from the viscera, when the temperature is increased from 70-80°C. The extracts are than analyzed through Gas chromatography using FID detector and positive results with 86% recovery of the fenvalerate are obtained.

Deshpande and Srivastava, (2016) - has suggested a method for extraction of pesticides from the tissues using QuEChERS method (quick, easy, cheap, effective, rugged and safe) which involves the cutting of the biological tissue into small fine pieces followed by its treatment with acidified acetonitrile solution along with the addition of 2ml of water in order to achieve the desired consistency and to remove the tissue dryness. The sample is placed in the extraction tube of SampliQ QuEChERS kit and allowed to sonicate for 5 minutes which is later treated with extraction salts (magnesium sulfate) which carries out the extraction. The sample tube is then shaken vigorously and centrifuged for about 4 minutes at 4000rpm to get a clear upper layer of supernatant which is again subjected to same treatment which involves centrifugation for 3 minutes in the SampliQ QuEChERS dispersive Solid Phase Extraction (SPE) tube to achieve a clear liquid extract. Deshpande and Srivastava has also extracted the pesticides from the body fluids (blood) using QuEChERS method by diluting 0.5ml of blood with distilled water in a SPE extraction tube containing 0.5 g pre-packed extraction preparation and a stainless steel bead. The spiking of the blood sample is done with the pesticide along with the addition of 2 ml acidified ACN (1% acetic acid/ACN), followed by vigorous shaking of the tube for a minute and allowed to centrifuge at 4000 rpm for 5 min. The supernatant obtained is then transfer to centrifuge tube after containing SPE sorbent, shaken vigorously and again centrifuged at 4000 rpm for 1 min so as to get a clear liquid extract. These extracts of both tissue and blood are then analyzed using high performance liquid chromatography. QuEChERS sample preparation reduces potential cross contamination of samples thereby facilitating rapid

and efficient analyzes of a large number of samples with an ordinary chromatographic instrumentation.

Discussion and Conclusion

The method of extraction applied for the pesticides analysis depends on the type of solvent or solvent mixture used, chemical and physical properties of the insecticide, the type of substrate (matrix) from which it is isolated and the method of estimation to be employed.

Stalling, et al, (1979); Ballschmiter *et al*, (1981); Mes, (1984); Srivastava and Yadav, (2014) – has carried out the liquid liquid extraction and steam distillation of pesticide using various polar solvents but the results obtained are not satisfactorily as the percentage of recovery is not much.

Wells, (1988) - conducted the analysis of pesticides (organochlorines) using Soxhlet extraction method but the results of the study showed that other co extracts are also come in the extracts as contaminants.

Repetto *et al*, (2001); Ueyama *et al*, (2006) – performed the solid phase extraction of the large number of pesticides using reverse phase medium C18, and derivatization Solid phase extraction using pentafluorobenzylbromide. The extracts obtained after clean-up are analyzed with gas chromatography and Gas chromatography Mass spectrometry (GC-MS) which showed that the differences occurred in their limit of detection and also in the recovery rate.

Lanjewar *et al*, (2014) - used accelerated solvent extraction method for the organophosphorus pesticides isolation from the biological samples and found that results of the study are encouraging as the detection upto minimum amounts along with high recovery percentage because the extracts are free from the matrix contaminants.

Chutke *et al*, (2016) – applied accelerated solvent extraction method for the isolation of fenvalerate (pyrethroid) followed by its determination using gas chromatography and found that the pesticide even subjected to higher temperature and pressure does not gets decomposed with 86% recovery.

Deshpande and Srivastava, (2016) – used QuEChERS method for the extraction of the pesticides from the biological samples and leads to the conclusion that the samples obtained are free from the matrix contaminants because of the efficiency of the method applied.

Extraction of pesticides from the biological matrix like viscera, tissues or body fluids through liquid liquid extraction or solid phase extraction methods requires large volume of solvent and are more time consuming therefore development in these extraction procedure using accelerated solvent extraction or other methods are considered to be better than conventional liquid liquid extraction and solid phase extraction method methods because of its less requirement of solvents, easy preparation of sample, less time consumption and greater efficiency for isolation and separation. QuEChERS sample preparation method can also be utilized for extraction of pesticides as this method reduces potential of cross contamination of samples and carries the rapid and efficient analysis of a large number of samples which can later detected with chromatographic methods. So, according to this paper the extraction of pesticides from the biological samples using Accelerated Solvent Extraction method and QuEChERS method are supposed to be the optimum methods as the results obtained are with maximum % recovery and also free from the interferences of matrix contaminants.

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Age Estimation of a Dried Bloodstain Using Different Techniques – A Review Article

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Abstract:

Bloodstains can be a crucial evidence in reconstructing the events that would have occurred during the crime. This information always strengthens and verifies the statement of witness and also narrow down the number of suspects. Currently there are many techniques available to establish the age of the bloodstains. However, there is no single reliable method which can be used in the field of forensic science to determine the exact time elapsed since the crime was committed. This review paper summarizes also the important techniques that have been used by many scientists for the determination of age of a blood stains at different conditions. By analyzing all the techniques one can select the best suited technique for his analyses, as different techniques have different accuracy rate, in addition to this some are destructive and some are nondestructive techniques. Therefore on comparing all these techniques one can able to find the best method according to the various environmental conditions and thus contributing the important role in investigation and for the court of law.

Key Words: *Age estimation, Blood stains, skeletonized stains, Hemoglobin, HPLC, Electron Paramagnetic Resonance Spectroscopy, Reflectance Spectroscopy, Atomic Force Microscopy, Hyper Spectral Image Analysis, Electron Spin Resonance Spectroscopy, Oxygen electrode.*

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Introduction

Blood is most commonly and frequently found at the crime scene and is the most important and useful physical evidence for the forensic investigators (Li et.al. 2011). This evidence is used for the DNA-profiling to authenticate the suspect's identity and its pattern reveals the direction of spattering in order to reconstruct the crime. Blood pool can easily be collected, preserved and examined by the analyst but sometimes investigators encounters the dried blood stains also. These dried bloodstains have a great value when used for the determination of age so as to find out the relative time of the trauma or bleeding because this information may help the investigators to determine, whether the stain is related to a crime and also the approximate time when the crime was committed (Edelman et.al. 2012). The blood begins to dry whenever it's exposed to the external environment and the time required by it is directly proportional to the size, volume, nature of the surface and the involvement of other factors. Under normal condition of temperature and humidity the small, lighter and thin bloodstain pattern on non-porous surfaces dries easily and doesn't take much time (Within few minutes), while under same conditions, the greater size or large volume blood stains requires longer period of time to get dry. The drying of blood stains begins around the periphery and then proceeds towards the central portion. The flaking central portion and the intact periphery or the rim of dried blood stain produces a characteristic skeletonized bloodstain. Age estimation of blood stain provides valuable and significant information and therefore found to be one of the important application during crime scene investigation (Inoue et. al. 1992).

Ageing of blood can be determined on the basis of color changes with the passage of time. As Fresh blood usually bright red in color because of the presence of oxyhemoglobin, after few minutes of deposition it appears dark red to bluish brown color because of the conversion of Hemoglobin (Hb) to Met-hemoglobin (met-Hb) and ultimately after few hours it appears dark brown color due to presence of hemichrome. So oxidation of HbO_2 results into the color change of blood which can be used for age determination of blood stain (Bremmer, 2012). This method is not reliable because we cannot find the accurate time of deposition as the result by visual analysis can vary person to person therefore to authenticate the results, different scientists developed different techniques to achieve the best results.

It is reported that, for more than 100 years many scientists used several methods and techniques for the determination of age from dried blood stain and they also proved that these methods are reliable and can be applicable for the forensic purposes (Li et.al. 2011). The various compounds present in a blood stain and depending of that there are numerous methods that have been established for this purpose, some of them are electron paramagnetic resonance spectroscopy, reflectance spectroscopy, atomic force microscopy, oxygen electrode, hyper spectral image analysis, electron spin resonance spectroscopy etc. (Inoue et. al. 1992).

The aim of this study is to summarize all the methods, techniques, time duration (up to which it provides correct result) and its reliability which is used by different scientist for the age determination of blood stains. This review study also evaluates the imprecision and unreliable results of many techniques and also covers the recent development and the future challenges and other factor that influences the result.

Review and Literature

Patterson (1960) worked on a well-known fact that the blood changes its color from red to brown with respect to time but there was no authentic literature for the rate at which these changes occurred. There are methods such as spectrophotometric analysis to determine the color but it was found to be destructive and its results was not accurate as it is altered due to the dirt or dye which comes in addition with the stain during extraction from the surface. He also found that sometimes extraction procedure also accelerates the color change of the stain. So to overcome these problems he used photoelectric colorimeter of high sensitivity and accuracy. This method was a non-Destructive method and gives results based on the surface color of the bloodstain, the reading provided by colorimeter can easily be converted to the C.I.E system of color specification. After performing experiment he observed that the greater part of the change in blood stain takes place within first three days after that the changes are minimal. Finally after analyzing the results he concluded that the temperature, humidity and lighting plays an important role and have a great effect on rate of color change.

Sakurai et.al. (1989) used Electron Spin Resonance technique to date human blood samples. This method uses the fact that the free radicals are generated when hemoproteins such as hemoglobin and myoglobin denatures in the blood stain due to the natural radiations. To investigate these proteins levels in the blood stain with respect to time he used this method. During the examination he observed peaks/signals, resulted due to the presence of some paramagnetic species such as Ferric Species or free radicals and the spectra of these signals was recorded at liquid nitrogen

temperature (77K) in a JES FE-IXG Spectrometer for about 270 days. After analyzing the results they concluded that this technique provides good results up to 270 days further more the better correlation with the time was found up to 120 days. Therefore ESR is a useful technique to determine the age of the blood stain.

Inoue et. al. (1992) for the first time utilizes the reverse phase high performance liquid chromatography (HPLC) for age estimation of blood stains. The method took advantage of the fact that the ratio of alpha-globin to heme is gradually decreases with time and therefore the peak area detected from the extracts of dried blood increases with time on chromatogram. It was found that this method was applicable up to 20 weeks old blood stains after that the precision gradually decreases due to the extra peaks appeared before and after the major peaks.

Matsuoka et. al. (1995) estimated the age of bloodstain by using the oxygen electrode. In this technique the oxygen content in the blood was determined by using the oxygen electrode which is immersed in water and the total hemoglobin was determined by colorimetry. Then the ratio of oxyhemoglobin to the total hemoglobin was used for estimating the age. They monitored the results for about 10 days and found that at different temperatures the decay rate of oxyhemoglobin (HbO_2**)** is different.

Anderson et. al. (2005) used real time reverse transcriptase PCR to measure the ratios between different types of mRNA i.e mRNA and rRNA (18 S rRNA to b-actin mRNA). This method concludes that, as the blood dried over a period of time, the level of these mRNA also changes in a linear fashion under controlled conditions. The time period over which one can get successful results by this method was found to be upto150 days.

Fujita et. al. (2005) used electron paramagnetic resonance (EPR) technique for the purpose of estimating the age of the blood stain. This technique measures the amount of hemoproteins which denatured under controlled environment. This method based on four major components i.e. ferric high spin, ferric non heme, ferric low spin and free radicals produced in blood stain and shows respective four striking EPR signals as g = 6.2 (g6), 4.3 (g4), 2.27 (H) and 2.005 (R) out of which g6 represents the level of met-Hb whereas H represents Hemichrome level (HC) in the blood. They found that the age of a bloodstain could be determined by the ratio of H/g4 signal. By using this criteria they got positive or suitable results for the dried blood stains which are not older than two months, beyond this period it is impossible to determine the exact age of blood stain.

Strasser et. al. (2007) presents a new tool for determining the age of bloodstains. They used Atomic Force microscopy to get the high resolution imaging of erythrocytes (RBC) of blood samples. This method was used to detect the changes, occurred in the elasticity of the erythrocytes as the function of time. Here the high resolution microscope consists of a cantilever and its tip (nanoindentor) scans the surface of the blood and provides a nanometer resolution image. The elasticity were recorded based on the Force- distance curves on different areas of a blood stain. After analyzing all the results they found that the elasticity of the blood was decreased with time or age of the sample.

Hanson and Ballantyne (2010) developed a method to estimate the time since deposition of bloodstain by the help of UV-VIS spectrophotometric analysis of hemoglobin. This method measures the wavelength of hemoglobin with maximal absorption around 412 nm but after detailed study of Soret band of hemoglobin they found that there was a blue shift (toward the shorter wavelength) with the increase in age of the bloodstain. This shifts can be easily distinguish between the blood that were deposited minutes, hours, days and weeks.

Li et.al. (2011) described a novel method for age determination of dried bloodstain. They used reflectance spectroscopy, a non-destructive method. It involves the use of micro spectrophotometer (MSP) that measures the visible reflectance spectrum of hemoglobin component present in the blood, deposited on a white tile. They monitored the results up to 37 days and the wavelength at which the spectra was generated were 442nm and 585nm under controlled conditions. To overcome the effects of baseline variation and sample scattering the spectra were preprocessed and to test the accuracy of results a supervised statistical classification model (leave- one-out-cross validation) was used.

Bremmer et. al. (2011) proposed diffuse reflectance spectroscopy (DRS) method to estimate the age of blood stains by determining the optical property of material. This method uses the advantage of the fact that the color of bloodstain changes with time from red to brown. The changed color can be quantified by optical spectroscopy. They have determined the levels of three major derivatives of hemoglobin in the blood such as oxyhemoglobin, met- hemoglobin and hemichrome as its fraction also changes with time. They found the unique combinations of these three component all the time in the blood samples over the period of 60 days.

Edelman et. al. (2012) used hyper spectral imaging to evaluate the feasibility of this technique for the

determination of age of blood stains. In this method they recorded the visible reflectance spectra of bloodstains and this spectra comprised of relative fractions of oxyhemoglobin, Met- hemoglobin and hemichrome with were later compared with the reference dataset. The results were analyzed up to 200 days. By this study it was found that the absolute error of age estimation increased with that of time. Further, to test the practical applicability of this method they simulated a crime scene where the number of bloodstains were deposited of several ages.

Li et.al. (2013) used visible wavelength hyper spectral image analysis to determine the age of dried bloodstain up to 30 days of time interval. In this method they generate the hyper spectral image of the dried blood stain and then selected some reflected spectra within the image which were then subjected to spectral preprocessing followed by linear discriminant analysis. This analysis was made under controlled conditions with an overall error of ± 1.17 days.

Discussion and Conclusion

(Patterson, 1960) first time used the reflectance measurements to estimate the age by using the spectrophotometer. (Bremmer et.al., 2011) also worked on reflectance spectroscopy over a period of 60 days under controlled conditions. The results obtained shows that they could able to find the correct age up to 55 days with the uncertainty of 14 days. (Inoue, 1992) worked on HPLC technique found this method suitable for the determination of age up to 20 weeks old bloodstains. This was the first time when some use this technique for age estimation. (Fujita et.al., 2005) used electron paramagnetic resonance spectroscopy, a non-destructive method for estimating the age up to 270 days under controlled environment. The results give a linear correlation of denatured proteins with the increased age but the accuracy of results found uncertain if the blood was influenced by or exposed to environmental factors such a absorbents, fluctuated temperature or sunlight. (Anderson et. al., 2005) worked on different component of blood i.e. the mRNA levels which gradually changes with the time. They found that the age of blood stain could be obtained up to 150 days by using real time reverse transcriptase PCR. This method offers the following advantages over other approaches such as enhanced detectability of small samples, simultaneous isolation of DNA and RNA from the same sample. (Hanson et. al.,2010) used UV-VIS spectrophotometer and found that the blue shift of the hemoglobin changes with the increase of time and directly influence by the ambient relative humidity and temperature. The method was extremely sensitive as it requires only 1µl of dried blood stain for analysis. (Edelman, 2012) used hyper spectral imaging system to estimate the age of bloodstains over the period of 200 days but they found that this technique gives absolute error rate with increased age of blood.

After analyzing all the papers it was found that there are numerous techniques have been used for age determination of bloodstains and all techniques are still in the experimental phase in addition with the inaccurate age estimation after a limit. On comparing all the techniques it was found that RNA method can be used for long term determination as compare to other techniques and the least destructive or invasive technique was Reflectance spectroscopy as it involves light source and a spectrometer which never destroys the sample. In addition to this the method was comparatively economical and gives good results for the samples on white background. The samples on colored background can be analyzed by hyper spectral imaging. HPLC and oxygen electrode method can be used for short term old bloodstains.

Some techniques are sensitive to estimate the age of bloodstains for short term changes and some are for long term changes. All the applied techniques, used only those blood samples which were dried at controlled conditions but as we know the results could be affected or influenced by environmental factors. So there is a need for further improvement which could provide highly précised and accurate results even if the bloodstains dried under different conditions. It is important to investigate the effect of other variables such as temperature, humidity, illumination, stain thickness and substrate so as to implement its use into forensic practice and eventually in court.

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