Quantification of Predetermined Components of Human Body Odour in Air Matrices by Gas Chromatography

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Abstract— **This paper presents, a strategy to identify persons based on measurement of human body odour from Human Sweat. To Approach this concept, the common volatile organic compounds (VOC) determined in the sweat of person of age 30 & above by using Master GC-Headspace Technique. The proposed method was capable to identify persons either with or without using deodorant irrespective of their eating habits.**

Keywords—
Odour, Head Space, Linearity, Reproducibility, Standard solution, Gas Chromatography

I. Introduction

The idea of distinguishing people by their odour is not a new concept. Trained dogs are routinely deployed by security and law enforcement agencies for forensic investigations and identification of a person committing crime. Odour is used by animals to recognize each other. People can distinguish the scent of different individuals, especially if they are unrelated or have different diets, and can recognize their own and their mate's scent [1] [4] [5] [7] [9].

Human scent has in abundance, the volatile organic compounds determined to be in the headspace above scent samples; however, other substances may contribute to human odour. The individual body odour of humans are determined by several factors that are either stable over time (genetic factors) or vary with environmental or internal conditions. For an individual identification by human scent, the primary odour has constituents that are stable over time and diverse people. Thus, body odour is one of the physical characteristics of a human that can be used to identify people.

Compounds present in male [10] and female [11] axillary secretion extracts that contained the characteristic odours present in the axillary region have been isolated and identified. These analyses showed the presence of several C6-C10 straight chains, branched, and unsaturated acids, and the major odour-causing compound was determined to be (E)-3-methyl-2-hexenoic acid. Other important odour contributors were terminally unsaturated acids, 2-methyl C6-C10 acids, and 4-ethyl C5-C11 acids. Short-chain fatty acids have also been extracted from sweat samples obtained from feet (Kanda et al. 1990)[2]. Olfactory evaluation by humans of 1000 ppm solutions of short-chain acids (C2-C9) showed that each short-chain fatty acid resembled either foot or axillary odour. Short-chain acids that resembled axillary odour tended to be higher in carbon number than those that resembled foot odour. Research has been done to determine the applicability of pattern recognition in analyzing and interpreting gas chromatograms produced from the analysis of human sweat [8]. Human odour components have been studied through headspace GC-MS for compounds specific to age [6]. Compound classes such as hydrocarbons, alcohols, acids, ketones, and aldehydes were present in human odour. This study also presented 2-nonenal as a compound that is only present in the odour of individuals over 40 year of age. 2-Nonenal, as well as other aldehydes, was produced through oxidative degradation of mono-saturated fatty acids, such as palmitoleic acid and vaccenic acid.[12]

II. Experimental Methodology

Samples were collected from a 30 year male subject by using absorbent pad collection, the sterile 2x2 gauge pad was placed over the site and covered with paraffin wax film to prevent evaporation. After collection it was stored in 10mL glass vial, from which sample was desorbed by suitable solvent.[13]The sample was closed in a vessel until the volatile components reach equilibrium between the sample and the gas volume above, i.e., the so called "headspace". An aliquot of the headspace was sampled and introduced into a gas chromatographic (GC) column for analysis.[14]

Standard solution of common axillary volatile organic component as acetone, acetic acid, benzaldehyde, ethanol, hexanal, heptanal, nonanal, n-octanoic acid, n-decanoic acid, n- nonanoic acid and phenol was prepared according to literature to get final concentration of each component 9.09%, 4 µl of this was then injected in Master GC – FID instrument, connected to a headspace auto-sampler, which was equipped with a flame ionization detector (FID). The SSL injector was used at 250°C to introduce the samples in column of DN-624(60m x 0.32mm x 1.8µ) and Nitrogen was used as carrier gas at constant flow rate 2.0mL/min.. The GC conditions were as follow: oven temperature 50° C for 5 min, increased at 10° C/ min to 230 °C held for 17 mins, detector temperature 250° C, the injection mode was split with a split ratio of 1:15. Master SHS conditions were as follow: oven temperature 220° C, incubation time 30 mins, valve temperature 250°C, transfer line temperature 250 °C and shaking was fast.

Chromatogram was plotted between voltage vs time fig. 1 shows the chromatogram of 11 standards at 9.09%, from which it is clear that ethanol was eluted first and give retention time 6.7 min and highest retention time was observed with n-decanoic acid. The table I shows the detail of all 11 standards with their respective retention time and concentration.

TABLE I
STANDARD WITH THEIR RETENTION TIME

Sr.	Component Name	Boiling Pt.	Concentration	Retention Time
1	Hexanal	119-124°C	9.09%	15.17
2	Heptanal	152.8°C	9.09%	17.34
3	Nonanal	195°C	9.09%	21.05
4	Acetone	56-57°C	9.09%	7.38
5	Benzaldehyde	178.1°C	9.09%	19.25
6	Ethanol	78.37°C	9.09%	6.7
7	Phenol	181.7°C	9.09%	20.41
8	Acetic Acid	118-119°C	9.09%	11.76
9	n-Octanoic Acid	239.7°C	9.09%	22.62
10	n- Nonanoic Acid	254°C	9.09%	24.12
11	n- Decanoic Acid	269°C	9.09%	25.65

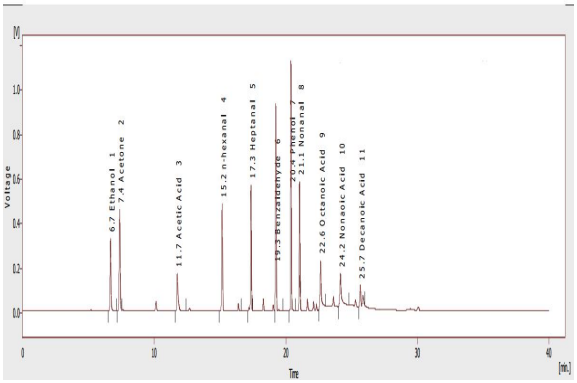

Figure 1: Chromatogram of the Standard solution

To validate method, the linearity and reproducibility were done using standard solution; selection of standard was done randomly. All analytical method validation was carried out as per official guidelines.

III. LINEARITY

Linearity of the method was determined by doing the injection of standards over the range of 2 µl to 6µl. the standard were spiked under the optimal headspace and chromatographic condition, the calibration curve was prepared by plotting relative peak area of the analytes against the analytes concentration. The correlation coefficient (r2) value for all standards were found to be 0.999, which showed that concentration of analytes and area of peak was in linear relationship and the calibration curve was linear and within the range(Fig. 2). Table II shows amount of analyte spiked and their respective area of peak.

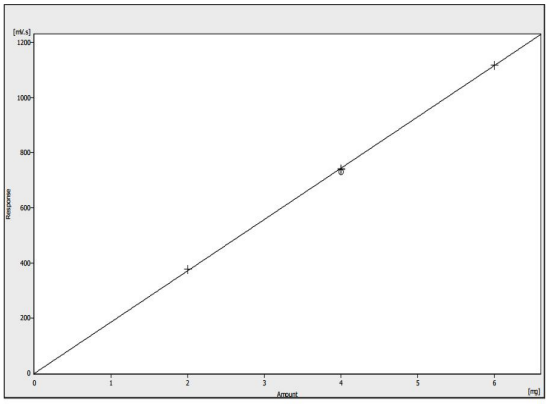

Figure 2: Calibration Curve of Acetic Acid.

Correlation Factor =0.9999381 which is acceptable.

TABLE III
SAMPLE DETAIL FOR LINEARITY

Vial No.	Amount Spiked	Area(mV)
1	2µl	378.1639
2	4 µl	742.0089
3	6 µl	1116.636

IV. REPRODUCIBILITY

Reproducibility was determined by spiking the standard with concentration at the mid point of the calibration curve and reported as a coefficient of variation. Method was optimized and expressed as the relative standard deviation (RSD), obtained from the determination of the three same samples of 4 µl. The RSD was found to be less than one which is satisfactory. The result is shown in table III and Fig. 3.

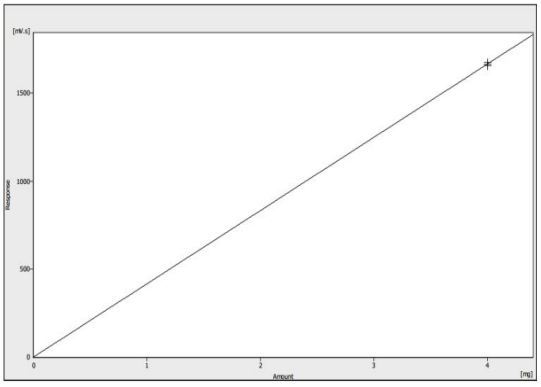

Fig 3: Reproducibility curve of n-Hexanal.

TABLE IIIII
SAMPLE DETAIL FOR LINEARITY

Chromatogram No	Amount	Area (mV)	Retention Time
1	4 µl	1468.2291	15.168
2	4 µl	1467.4080	15.172
3	4 µl	1466.6338	15.160
	RSD%	0.054365%	0.040286%

Linearity and precision results demonstrate that the method is precise and accurate.

V. Sample Analysis

A 60m x 0.32mm wide columnwas used, with nitrogen as carrier gas with a 2 ml/min. The column temperature was initially maintained at 50°C for 5 min. , was further raised at rate of 10°C/min., finally raised at 230°C and maintained for 17 min.

Aliqous 4µl sample was injected in headspace mode with equilibrium time for 30 min.. The transfer rate line temperature was 250°C. The chromatogram was recorded and presented in Fig 4& Table IV.

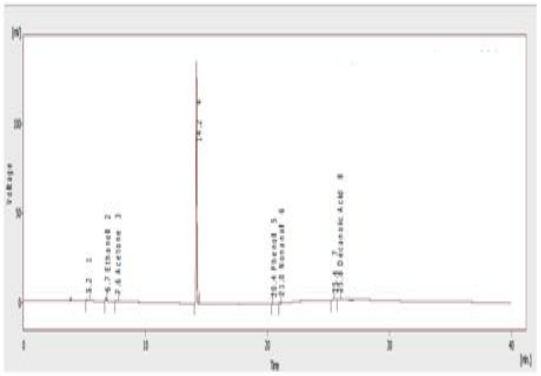

Fig 4:Chromatogram of Sweat Sample

VI. Result & Discussion

A Gas chromatographic method was developed for the analysis of sweat sample with DN-624 column by headspace injection method. The method showed good separation and resolution between the peaks of the volatile organic compounds of sweat of the subject [15]. The chromatogram was shown in Fig 4 and retention time, area of peak, amount of different VOCs is shown in table IV.

The retention time were recorded as ethanol 6.73min., acetone7.3 min, phenol 20.37min., nonanal 21.0 min, deconoin acid 25.81 min. By comparing all these with the data of standard, it was found that the common VOCs are found in subject irrespective of their gender, age and use of deodorants [12,14].

VII. Conclusion

Over the solid phase microextraction (SPME), the headspace GC permits the determination of volatile present in an essentially non volatile matrix , which may require sample extraction or preparation and the selection of equilibrium conditions mainly temperature, so that the volatile concentration can be measured in the headspace, make the easier determination of trace concentration in the sample.[16]

The one unknown component in sweat sample at retention time 14.2 min. was obtained, which is present in very large amount. It can be an artificial chemical compound such as deodorant used by the subject and the same can be determined using MS technique for further research. Identification of such chemical compound, may act as individual fingerprints, which will be used as the method for identifying or characterizing the subjects and will become an important tool in field of forensic science.

Headspace GC is a very promising method in the analysis of volatile components with good reliability and reproducibility. The result obtained by this technique is satisfactory with a good resolution, accuracy and precision of analytes tested.

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