

# Prospective Chemical Profiling of Human Apocrine Sweat

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Received: 16<sup>th</sup> Dec, 2025; Revised: 8<sup>th</sup> Feb 2026; Accepted: 12<sup>th</sup> Feb, 2026; Available Online: 28<sup>th</sup> Feb, 2026

## ABSTRACT

Body odor is found to be unique to every individual. Body odor is mainly caused by the bacterial action on the sweat present on the skin surface and the human axilla is the main source in causing body odor. This study is the preliminary examination of organic components, mainly primary body odorant, present in the apocrine secretions. Trans-3-methyl-2-hexenoic acid which is one of the primary odorants was found in the human apocrine sweat and was earlier found in the axillary sweat of Caucasians and some Asians and no female samples contained this short-chain fatty acid. For this study, 20 Gujarati male volunteers of age between 18 and 26 provided the apocrine secretions on 2 to 3 random days. The samples were collected and analyzed using FTIR for the functional groups present in the glutaminy-conjugate of trans-3-methyl-2-hexenoic acid. The functional groups present in the same acid were found in the apocrine sweat of the male volunteer despite the diet intake, surrounding temperature, and lifestyle changes. On this basis, the sweat found on the crime scene can be distinguished as eccrine and apocrine, as same primary odorant is not found in the eccrine sweat as well as to some extent, the male and female apocrine sweat can be distinguished.

**Keywords:** Apocrine sweat, Crime scene, TMHA, FTIR Spectroscopy, Chemical Profiling, Forensic Science

**How to cite this article:** Abrol V, Ghadge D, Babu GR. Prospective Chemical Profiling of Human Apocrine Sweat. *Int J Drug Deliv Technol.* 2026;16(17s): 188-192. DOI: 10.25258/ijddt.16.17s.23

**Source of support:** Nil.

**Conflict of interest:** None

## INTRODUCTION

Apocrine glands are present in the hairy parts [1] of axillary region and the genitals [2] [3]. They are inactive until puberty [1]. The sweat volatiles of an individual have specific pattern which make them discriminate from other people [4]. Although it is found that different body organs such as the neck, head, and underarm has different bacteria at different ages like teenage and pre-pubescent, and have different odor intensities, the underarm has maximum number of bacteria [5] [6] [7]. Body odor can even be traced back into Roman Empire, which had bath houses and the gloves of Queen Elizabeth I used to have perfume in it. [3] Since the last 40 years, antiperspirants and deodorants have been used to overcome it [3] [7]. Overall scent of a person is the combination of his secretions and excretions. [3] It influences the behavior of a person [6]. It can be collected on an adsorbent surface [8] Human axilla also known as 'scent organ' [9] produces body odor [3] because of the microbiota [6] [10] present on its surface, hormones [6] plays a significant role in body odor production. Bacteria break down the precursor-odorant complex, cleaves the covalent bonds and attach acid molecules to the precursors [6]. Hormones control production of glands in the axilla [6]. It is diverse in different race and at different time [3] also differ with sex and age [6]. Along with axilla, bacterias are found in nasal

vestibule, the scalp, face, limbs, external ear, perineum and the groin [6].

Hominis [5] [10], lugdunesis [10], epidermidis [5], and haemolyticus [10] of Staphylococcus family are the major odor causing bacteria in the armpit. Corynebacterium [11] and Cutibacterium [11] along with Staphylococcus [11] are the highly populated bacteria [7] with the help of enzymes [7] convert odorless [3] [6] [7] and oily fluid [1] [5] into malodorous. Acids such as isovaleric acid [5], acetic acid [5], sulfanyls [5] and volatile steroids such as androstenone [12](but is not a main component [7]), androstadienone and androstenol [12], 5 $\alpha$ -androst-16-en-3-one [6] [7], 5 $\alpha$ -androst-16-en-3 $\alpha$ -ol [7], 3-methyl-2-hexenoic acid [7], a major protein called apolipoprotein D [7] Esters (ethyl-2-methylpropanoate and ethyl butanoate), ketones (1-hexen-3-one and 1-octen-3-one) and, in particular, aldehydes [(Z)-4-heptenal, octanal, (E)-2-octenal, methional, (Z)-2-nonenal, (E,Z)-2,6-nonadienal, (E,Z)-2,4-nonadienal, (E,E)-2,4-decadienal, and 4-methoxybenzaldehyde] were identified as primary odorants in the study done by Signe Munk, et.al [13]

Volatile Organic Compounds, very commonly known as VOCs as well as Volatile Fatty Acids (VFAs) [11] are the contributors of body odor. Few of them are 3-methyl-2-hexanoic acid(3M2H) which produces goat-like odor, 3-hydroxy-3-methylhexanoic acid(HMHA) produce cuminal

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like odor, strong fish-like odor is produced by trimethylaminuria, musty odor by phenylketonuria and hypermethioninemia causes odor to smell like boiled cabbage. [11] 2-nonenal suggests aging from the unpleasant odor' [2] Trans-3-methyl-2-hexenoic acid is the major contributor to axillary odor. [14] Techniques such as Solid Phase Microextraction (SPME) [2], SPME coupled with Head Space [15] are used for the extraction of VOCs. For the analysis of VOCs, Gas Chromatography/Mass Spectrometry [2] [15] [9] [16], Gas Chromatography- Flame Ionization Detector (GC-FID) [2] [4] [17], Gas Chromatography-Olfactometry [15], Liquid Chromatography-Tandem Mass Spectrometry [9] [18], H NMR Spectroscopy [19]

Reversed-phase High-Performance Liquid Chromatography along with UV detector has been used for analysis of sweat chloride [20] Body odor is being used for clinical diagnosis. [3] [6]. Trans-3-methyl-2-hexenoic acid is mainly found in the sweat of schizophrenic patients [3]. Chloride is found in patients with cystic fibrosis [3] Strong unusual odor is a primary symptom in epileptic patients, it is also observed in hidradenitis suppurativa, paranoia, phobias and organic lesions of CNS and there are numerous such diseases which are linked with a characteristic odor [3]. Mammalian Semiochemistry, also called Chemical Ecology, is another area that lies between biology and chemistry and studies pheromones that work as chemical signals between mammals [21]. It is also seen in highly endangered and one of the smallest primates, tamarins and marmosets, the New World monkeys from Callitrichid family [22] Pheromones are the volatile compounds which play an important role in maintaining the sexual relationships [6].

Leher Brisbin, Jr and Steven used three canines to distinguish their handler from other persons. They could identify the handlers from the scent of the body part which they were trained but could not identify the scent collected from other body parts [23] but this could happen because different body parts have different bacterias on them [5] FTIR spectroscopy is used to obtain structural and functional information of the compound analysed using it. [24] Fatty acid purification is often accomplished using SPE columns with a bonded aminopropyl stationary phase, which may be used to separate organic samples into three fractions: lipids of low to medium polarity, free fatty acids and a phospholipids fraction. [25]

## MATERIALS AND METHODS

### Materials

Petri dish, measuring cylinder, beakers (25ml, 50ml, 100ml), ethanol, distilled water, Rotatory Shaker,

Centrifuge, Concentrator, Weighing Balance, Filter paper (pore size 0.45  $\mu\text{m}$ ), micropipette.

## METHODOLOGY

### Sampling

Total 60 samples of 20 male volunteers from the state of Gujarat between age 18 and 26 were taken on 3 random days. Merc filter paper of 0.45  $\mu\text{m}$  pore size was used for sample collection. The subjects were asked to keep the filter paper in armpit area for 2 hours for random 3 days after bath. Subjects were abstained from using deodorants or antiperspirant and the filter paper was not touched barehanded.

Information of the subjects including name, date of birth, age, sex, height, weight, city/town, profession, diet type (veg or non-veg), smoking habit and alcohol consumption, body soap used to clean the armpit area, date, time and duration, temperature at the time of sample collection, the diet on and before the day of sample collection was collected.

### Sample Preparation

The sample containing filter paper was put in 10% 15ml ethanol and put on a Neuation iSHAK 3D-5 rock shaker for 2 hours at 98 rpm. The extracts were stored in falcon tubes in the refrigerator at  $-20^{\circ}\text{C}$ . The sample was centrifuged at 2500 rpm at  $26^{\circ}\text{C}$  for 6 min. The supernatant was taken for FTIR analysis.

### FTIR

Infrared (IR) spectroscopy is essential for the identification of molecular species and the quantification of their concentration in a given sample. This technique is non-invasive, indicating that it does not alter the sample while conducting the analysis. In a conventional IR spectroscopy configuration, the sample is exposed to infrared radiation, inducing molecular vibrations within the sample. The IR spectrum of the sample is acquired by quantifying the absorption or transmission of infrared light as a function of either wavelength or wavenumber. The Fourier-transform infrared (FTIR) spectra of sweat samples offer significant insights into the molecular composition of the sweat. Through the examination of FTIR spectra, it is possible to discern the molecular species that are present in sweat and ascertain their concentration. The FTIR spectra of all the sweat samples were obtained and studied wavenumber against transmittance in percentage.

## RESULTS

### FTIR Analysis of Axillary Sweat Samples

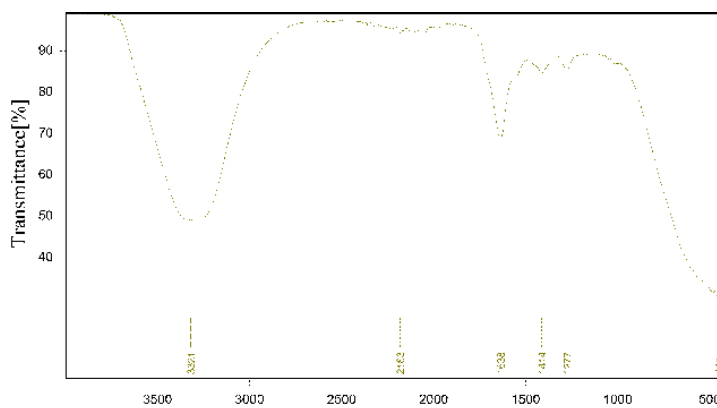


Figure 1 FTIR Graph of Sample 22

The broad and strong peak at 3321  $\text{cm}^{-1}$  shows the presence of intermolecular H bonding, the presence of alkyne group ( $\text{C}\equiv\text{C}$  stretching) can be seen at 2183, peak at wavenumber 1638 is the indication of imine or

conjugated alkene ( $\text{C}=\text{N}$ ), 1414 is the peak of compound containing sulfate  $\text{S}=\text{O}$ , aromatic amine( $\text{C}-\text{N}$ ) can be seen present at 1277.

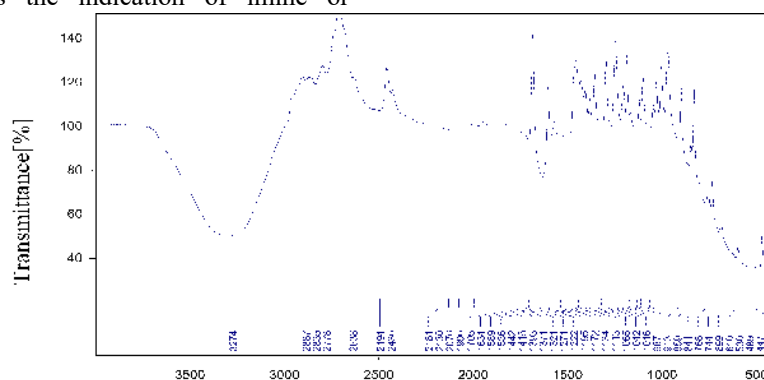


Figure 2 FTIR Graph of Sample 39

The broad and strong peak at 3274  $\text{cm}^{-1}$  shows the presence of intermolecular H bonding, presence of aliphatic ketone ( $\text{C}=\text{O}$  stretching) can be seen at 1706 peak at 2494  $\text{cm}^{-1}$  is unknown, peak at wavenumber 1636 is

the indication of imine or conjugated alkene ( $\text{C}=\text{N}$ ), 1415 is the peak of compound containing sulfate  $\text{S}=\text{O}$ , aromatic amine( $\text{C}-\text{N}$ ) can be seen present at 1271.

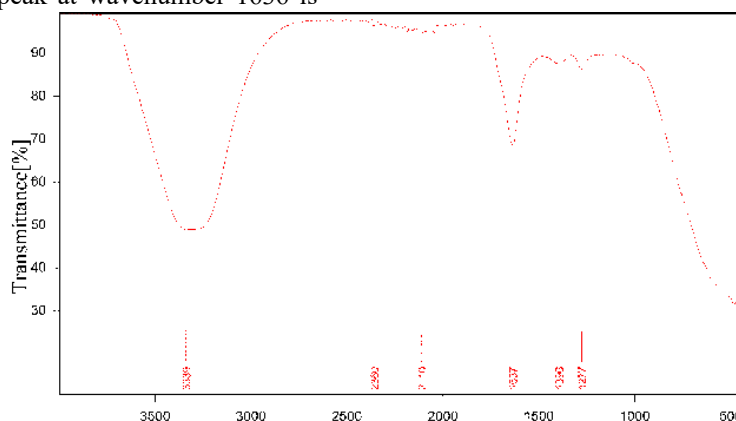


Figure 3 FTIR Graph of Sample 19

The broad and strong peak at 3339  $\text{cm}^{-1}$  shows the presence of intermolecular H bonding, the sharp peak at wavenumber 1637 is the indication of imine or conjugated alkene ( $\text{C}=\text{N}$ ), 1396 is the peak of a compound containing sulfate  $\text{S}=\text{O}$ , aromatic amine( $\text{C}-\text{N}$ ) can be seen present at 1277.

**DISCUSSION**

In Fourier Transform Infrared Spectroscopy (FTIR) peaks between 2300 and 4000  $\text{cm}^{-1}$  are frequently associated with vibrations brought on by stretching of hydroxyl( $-\text{OH}$ ) groups. This study aimed to determine the presence of trans-3-methyl-2-hexenoic acid which is primary odorant found in the apocrine sweat of a human being and to check

whether the presence of it differs with the lifestyle changes, diet, and other factors such as age. For the preliminary study of the primary odorant, Fourier Transform Infrared Spectroscopy was used and the values obtained in the graph of percent transmittance versus wavenumber was compared with the standard reference table of FTIR Values. Trans-3-methyl-2-hexenoic acid contains several functional groups including amide (CONH<sub>2</sub>), aliphatic ketone (C=O), imine or conjugated alkene (C=N), were found. Also, a weak peak of Sulfonic acid can be seen. Other than those functional groups two unknown peaks at 1485 and 1445 are found, these can be an instrumental error or an unknown compound might be present in the sample.

### CONCLUSION

The functional groups present in the Glutaminylyl conjugate of trans-3-methyl-2-hexenoic acid are present in the peaks obtained from FTIR analysis, so it can be said that one of the primary odorants, trans-3-methyl-2-hexenoic acid, is present in all of the male apocrine sweat samples. However, the quantitative analysis of those odorants is needed to individualise the secretor. That's why, for precise results, use of SPE for extraction and GC-MS or LC-MS for analysis is recommended.

### Conflict of Interest

There is no conflict of interest for this study.

### ACKNOWLEDGMENT

I express my heartfelt gratitude to those who gave me their valuable time and samples for the analysis.

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