RESEARCH PAPER

Molecular and Elemental Analysis of Thyroid Disorders through Eccrine Sweat

Dr. Vaishali Abro[1*], Srushti Raghavendra[1], Divyant Kataria Jain[2] and Dr. G. Rajesh Babu[3]

[1]Assistant Professor, School of Forensic Sciences, National Forensic Sciences University, Gandhinagar, Gujarat*
[1]Student, School of Forensic Sciences, National Forensic Sciences University, Gandhinagar, Gujarat
[2]Research Scholar, School of Doctoral Studies and Research, National Forensic Sciences University, Gandhinagar, Gujarat
[3]Professor, School of Medicolegal Studies, National Forensic Sciences University, Gandhinagar, Gujarat
[1]vaishaliabrol786@gmail.com, [1]srushtir21@gmail.com, [2]divyant.phdfs2370@nfsu.ac.in and [3]rajesh.babu@nfsu.ac.in*

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ABSTRACT
Sweat is a biological fluid mainly composed of water (99%), with several biomolecules, electrolytes, and metabolites. Its constitution includes water, elemental components (mostly sodium, chloride, and potassium), trace elements (calcium, magnesium, and zinc), and organic components such as small amounts of metabolites (urea, glucose, and lactate) as well as other compounds like cytokines and hormones. Hydration state, medication use, the effect of exercise, and the heat and humidity of the environment can lead to variation in the composition of sweat from person to person. The sweat glands play an important part in thermoregulation, also aiding in the excretion of metabolic wastes and toxins, including heavy metals and some organic compounds, from the body. This research explores sweat as a viable medium for the excretion of levothyroxine, a drug used to treat hypothyroidism, which is collected on filter paper and analysed. Levothyroxine was identified using energy-dispersive X-ray fluorescence and Fourier transform infrared spectroscopy. EDXRF generally gives sensitive elemental analysis, with FTIR indicating molecular structure, biological markers, and functional groups characterized by absorption spectra at a given sample concentration. This study presents a unique molecular signature from sweat that can offer a non-invasive method to use it as a potential biomarker in drug monitoring. Thus, the paper presents a thorough examination of the use of eccrine sweat as a diagnostic fluid for levothyroxine detection, in which the molecular profile of fingerprints may serve as a tool for personal identification and drug-use monitoring, paving the way for enhanced, non-invasive diagnostic methods in healthcare.

Keywords: Fingerprint Residue, Molecular Fingerprinting, Thyroid Disorder, Levothyroxine, Spectroscopy, Eccrine Sweat.

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1. INTRODUCTION

Fingerprints are the unique and permanent patterns formed by the friction ridges on the fingers of human. These patterns are formed during foetal development, making them permanent and a valuable source of evidence for personal identification and forensic investigations [1,2]. Each fingerprint consists of distinct ridge patterns and minutiae points that serve as identifiers. Beyond these ridge structures, the sweat glands present in the dermis layer of the skin contribute to the deposition of the latent prints, providing information on the chemical composition of sweat [3]. Sweat is produced predominantly by eccrine glands that are located on the palms and soles [4]. The composition of sweat gets affected under various physiological, metabolic, and environmental conditions; it can also serve as a diagnostic biofluid for health monitoring and forensic applications [5]. Studies have shown that the variation in the secretion of sweat based on factors such as heat acclimation [6] and aerobic training enhances the production of sweat. However, dehydration and hypovolemia can result in a reduction of sweat. Apart from these age is also considered as a factor that has an impact on the production of sweat [4].

Eccrine sweat glands are responsible for thermoregulation and excretion, that plays an important role in the deposition of fingerprints. The physiological functions and applications are understood through studies of sweat composition and secretion mechanisms [5]. Eccrine sweat is typically composed of more than 98% water and several organic and inorganic substances that can vary from person to person. Inorganic compounds include sodium, chloride, calcium, potassium, magnesium, iodide, copper, tin, mercury, lead, manganese, molybdenum, sulphur, and cobalt. Organic compounds include amino acids, proteins, glucose, urea, lactate, creatinine, vitamins, pyruvate, and uric acid [7]. The mechanism that influences the composition of eccrine sweat such as hydration status, electrolyte balance, and glandular activity is explained by [8]. Apocrine glands secrete milky sweat contains proteins and lipophilic chemicals, such as pheromones and steroids,

**Author for Correspondence: vaishaliabrol786@gmail.com*

that secrete solutions with much higher concentrations of protein and lipid than eccrine glands [9]. Whereas, Squalene, wax esters, triglycerides, and phospholipids are the primary components of sebum produced by sebaceous glands. Other components of sebum include glycerides, cholesterol, cholesterol esters, and free fatty acids [7]. Researchers also provided a comprehensive understanding of sweat gland physiology, emphasizing on how sweat secretion, reabsorption, and external contamination influence its composition, making it a valuable but complex biofluid for analysis. These studies emphasize on the growing potential of sweat analysis in pharmaceutical, forensic, and medical applications, highlighting the need for standardization and further research to optimize its diagnostic and analytical capabilities. [4, 8]

Sweat analysis has gained attention as a non-invasive diagnostic tool for detecting various substances, health conditions, and biomarkers. Studies have shown the effectiveness of analytical techniques such as EDXRF, SEM-EDX, FTIR, and LC-MS in analysing sweat composition. Demonstrating drug detection using colorimetric techniques has emphasized the role of sweat glands in excreting drugs [10] and utilizing LC-MS for measuring antipsychotic drugs in sweat has shown its potential for monitoring drug adherence [11]. [12] used FTIR spectroscopy for the quantitative detection of levothyroxine sodium, offering an alternative to HPLC in pharmaceutical analysis. FTIR and XRD were utilized to evaluate the drug uniformity of topiramate and levothyroxine sodium, by strengthening the chemometric approaches for quality control purposes [13]. Similarly, [14] explored the role of molecular mediators like PACAP (Pituitary adenylate cyclase activating polypeptide) that influenced the quantity and composition of the sweat produced. Spectrometric analysis of toxic elemental impurities such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As), using ED-XRF made it a valuable tool for ensuring drug safety and quality [15]. [16] detected elemental impurities in medicinal plants, where elements like Cu, Zn, Mn, and Se were detected using EDXRF. Furthermore, [17, 18] demonstrated how advanced techniques like XRF and SEM-EDX can uncover environmental trace and chemical elements embedded in sweat residues. Medical diagnostics have opened ways in the detection of cystic fibrosis through sweat analysis [19] while, Madoff, L. highlights the relation between altered sweat chloride levels and hypothyroidism [20] and Means MA identified cytological changes in eccrine sweat glands of patients diagnosed with hypothyroidism by performing biopsy.[21] Advancements in endocrinological diagnostics was discovered by inventing a non-invasive photodetector bases technique for evaluating thyroid functions through skin resistance [22]. Yang et.al engineered a soft, flexible microfluidic devices that adheres to the skin, capturing and analysing sweat in real-time, by monitoring of electrolytes, metabolites, and hormones, paving the way for continuous health monitoring. [5]

Analysis of fingerprint ridges has been used as an anthropological tool, as it provides demographic information, such as the determination of sex [23] [24], thereby increasing the significance of ridge analysis in personal identification by revealing the relationship between fingerprint ridge density and gender. Yarovenko et. al analysed the dactyloscopic and dermatoglyphic features of fingerprint ridge patterns in serial killers who committed crimes for mercenary motives, identifying how fingerprint ridge analysis can reveal behavioural and psychological traits in forensic investigations.[25] Consideration of both ridge patterns and minutiae features for matching of fingerprint ensured that both these features provide a higher accuracy and improved identification [1]. Liu et.al developed an algorithm for fast fingerprint classification by tracing ridge pattens streamlining the fingerprint identification process, and increasing its applicability in real-time forensic scenarios.[3]

Hypothyroidism is the most common thyroid disorder, which is characterized by insufficient production of thyroid hormone, affecting multiple organ systems, thermoregulation, and metabolic processes of the body [26]. Traditional methods for the diagnosis and management of hypothyroidism highly rely on serum analysis, which involves invasive drawing of blood to monitor hormonal levels and treatment [27]. Emerging studies indicate that sweat secreted from the eccrine glands could serve as an alternative non-invasive matrix for physiological monitoring [28]. Analysis of chemical constituents in sweat residue through molecular fingerprinting has enabled the detection of exogenous substances such as drugs, including therapeutic drugs like levothyroxine [29]. Levothyroxine is a synthetic form of T4 hormone that is used to treat hypothyroidism. Monitoring its presence in bodily fluids can evaluate the pharmacokinetics, compliance, and physiological response of the patient [12].

2. METHODOLOGY

2.1 Sweat Sample Collection

Sweat samples were collected from different patients of both genders, who were taking medication for diabetes, blood pressure or hypothyroid. Sweat samples were collected using a passive collection technique. Passive collection was performed by grooming the hands using ethanol/acetone and use sterile, absorbent patches Whatman filter paper, that was placed on to the participant's palm. The patches were left in place for a duration of 45-60 minutes to allow sufficient perspiration. After collection, the patches were immediately transferred into sterile zip lock covers and stored at 4°C or below to prevent degradation of components before analysis.

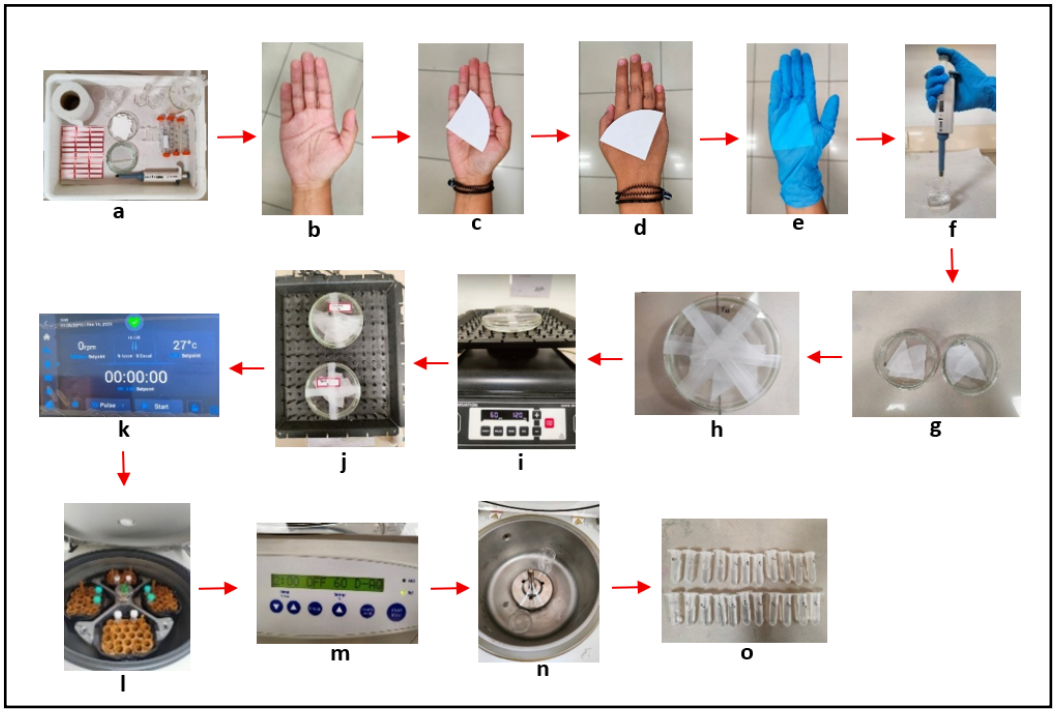


Figure 1: Methodology a. Materials required b. Groomed hands for sweat collection c. Collection of sweat samples. Collection of a sweat sample e. Gloves to increase perspiration and prevent contamination f. Solvent extraction (methanol + distilled water) g. Sweat samples in the solvent system h. Wrapped in paraffin strips i. Parameters set for rotatory shaker j. Samples placed on the rotatory shaker k. Parameters set for centrifugation l. Samples placed in the centrifuge m. Parameters set for concentration bath n. Samples placed in concentration bath o. Concentrated samples were transferred into Eppendorf tubes.

2.2 Sweat Extraction and Sample Preparation

The absorbed sweat was extracted from the filter paper patches using a liquid-liquid extraction approach. The solvent system was prepared by adding 13.5mL of distilled water and 1.5mL of methanol. In a sterilised petri dish, the filter paper is placed along with the prepared solvent system. The Petri dishes were sealed with paraffin wax strips before being placed on the rotary shaker to prevent spillage. The instrument was set to run at 60 RPM for 2 hours. Then the liquid was transferred into Falcon tubes using a micropipette. These tubes were then centrifuged at 4000 RPM at 27°C for 10 minutes. This was followed by transferring the sample into beakers and placing it in a concentration bath to concentrate the sample. The parameters that were set for the instrument were Daq (distilled aqueous) mode, for 2 hours at 60°C to concentrate the samples from 15 mL to 3 mL. Then the samples were collected in the Falcon tubes and stored at 4°C until analysis was done.

2.3 Analytical Techniques for Sweat Composition Analysis

2.3.1 Energy Dispersive X-ray Fluorescence (EDXRF)

The elemental analysis of sweat samples was performed using Energy Dispersive X-ray Fluorescence (EDXRF) to detect the presence of various elements. The filter paper samples were directly mounted onto the sample holder one by one and placed inside the EDXRF spectrometer. The emitted X-ray spectra were recorded and analysed to determine the elemental composition.

2.3.2 Implementation of EDXRF

The main power button is turned on. Then the UPS, CPU, monitor, and the Shimadzu EDXRF instrument are turned on. The software used for analysis was PCEDX-Pro. Once the software is opened, it needs to be initialized. The initialization process begins by clicking on the OK button. The detector gets ready by optimizing the temperature for working. The system asks to turn on the key for X-rays. Once the key is turned on, the system takes 15 minutes to standardize. Since the X-rays produce a large amount of heat, it is required to run the instrument in an air-conditioned room. Next, the system needs to be calibrated by using an Aluminium standard sample. Click on the Maintenance tab, followed by the instrument calibration option. Place the Al-standard in the analysis holder of the EDXRF and close the lid. To start the calibration, click on the start button on the system. It takes about 5 minutes to run. Once the calibration is done, the samples are ready to be analysed. Click on the Analysis tab on the screen and select the Easy group for the elemental analysis of the samples. After placing the sample in the holder, close the lid and click on the start button on the system. After the run, the results will be displayed on the screen, which can be saved in Excel format.

Table 1: EDXRF report of Standard medicine

Element	Result	Std.Dev.
Ca	33.233	[7.897]
P	26.023	[18.314]
Al	17.110	[45.935]
S	13.443	[10.515]
K	2.331	[7.639]
Fe	2.095	[1.703]
Cu	1.925	[1.159]
Mn	1.220	[1.973]
Cr	1.173	[2.370]
Zn	0.751	[1.001]
Co	0.697	[1.496]

Table 2: Average EDXRF report of Healthy Individuals (1 to 5)

Element	Average Result	Avg. Std. Dev.
Ca	38.14	1.79
K	21.83	3.22
P	15.10	4.44
Al	12.15	14.06
S	7.06	3.33
Fe	1.36	0.61
Ti	0.89	0.36
Cu	0.89	0.22
Mn	0.76	0.58
Cr	0.65	0.40
Zn	0.83	0.23
Co	0.38	0.41
Ag	0.31	0.28
Ni	0.23	0.09

Table 3: Average EDXRF report of Thyroid Individuals (1 to 20)

Element	Average Result	Avg.Std. Dev.
Ca	41.10	1.55
P	20.82	3.74
K	10.53	2.46
Al	14.87	8.98
S	9.06	1.39
Fe	1.55	0.56
Ti	0.88	0.74
Cu	0.96	0.28
Mn	0.90	0.40
Cr	0.75	0.50
Zn	0.30	0.15
Co	0.38	0.27
Ag	0.27	0.13
Si	5.04	1.97
Ni	0.37	0.24

2.3.3 Fourier Transform Infrared (FTIR) Spectrometer

Fourier Transform Infrared Spectrometer (FTIR) uses the Fourier transform technique in which the infrared spectrum of any material (solid, liquid, and gas) is obtained by recording the absorption or emission of the various materials. With the Fourier Transform Infrared Spectrometer (FTIR), one can get the details of molecular structure and composition of the molecule in much greater detail.

2.3.4 Implementation of ATR-FTIR spectroscopy

Switch on the power button, followed by turning on the CPU, monitor, and Jasco-FT-IR Spectrometer Model: FT-IR 4700 with ATR. The Spectra Manager Suite software was used for analysis. The sample holder must be cleaned with acetone before placing the sample. Then the holder should be cleaned with the solvent system prepared for extraction (Methanol + Distilled water). A few microliters of the solvent were added to the sample holder using a micropipette as a blank run. The screw on top of the holder is tightened and the instrument is closed. The sample is analysed by clicking on the blank button on the system. When the next sample needs to be analysed the instrument lid is opened and the screw is loosened and cleaned throughly with acetone. This needs to be done after every sample analysis. When the samples are placed, the sample button needs to be clicked for analysis. Once the run is complete, the graph is displayed on the system. Click on the Correction option, followed by Baseline, to make the baseline correction for the graph. If smoothening is required, click on correction and then the Smoothening option. After this is done, go to Processing and click on the Peak processing, followed by Peak find. The system shows the graph with the respective peaks and their wavelengths. Once this is done, the results are saved in the PDF format by clicking on the print option.

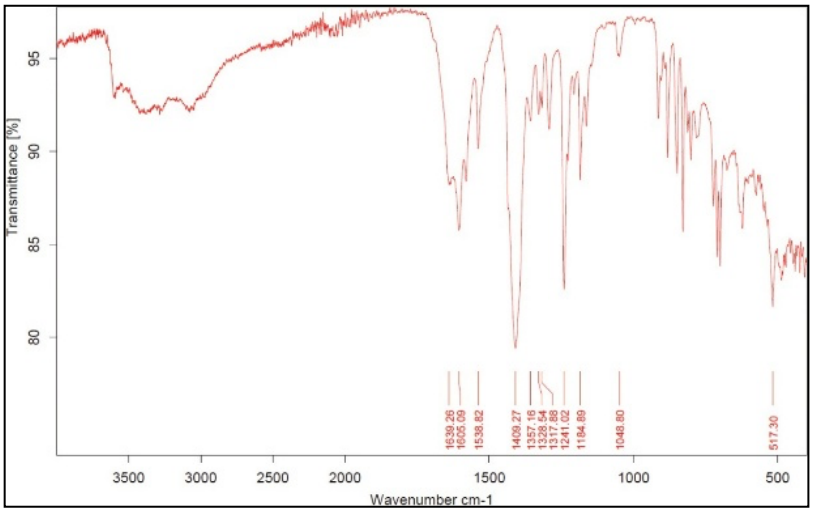


Figure 2: Standard graph of Levothyroxine Sodium

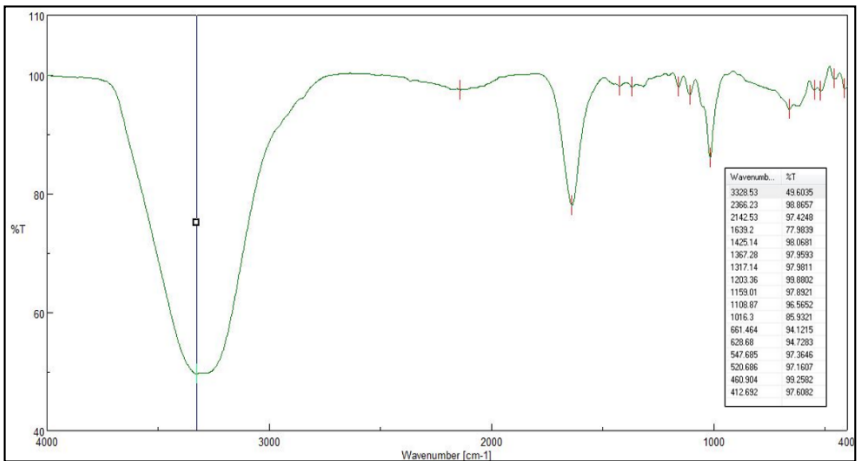
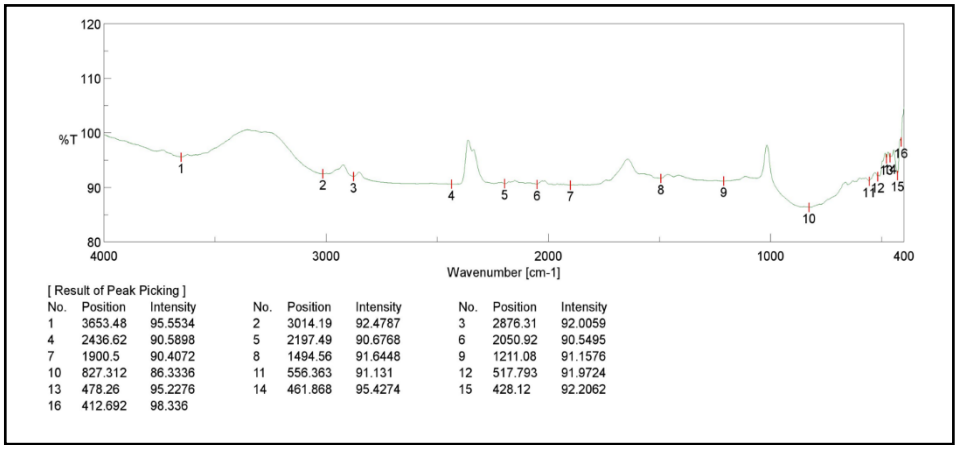


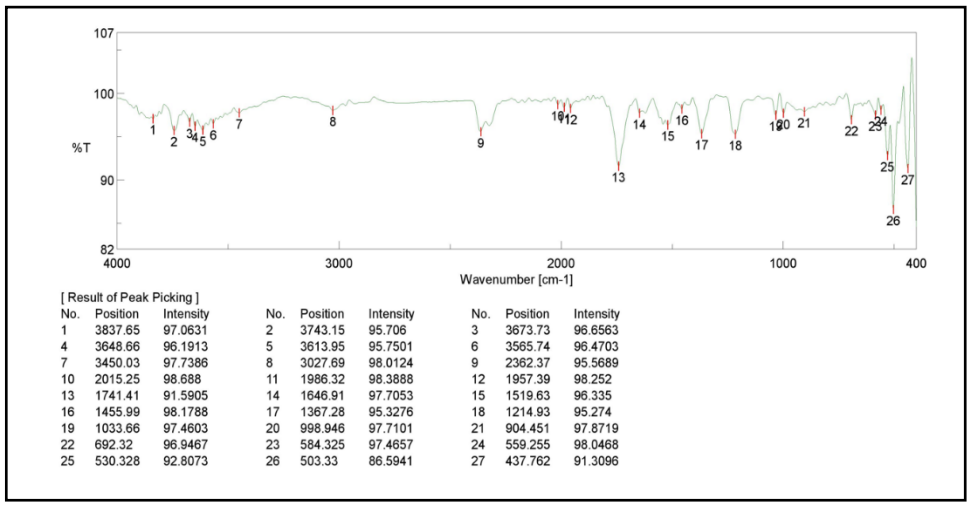
Figure 3: FTIR graph of Standard medicine



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3653.48	95.5534	2	3014.19	92.4787	3	2876.31	92.0059
4	2436.62	90.5898	5	2197.49	90.6768	6	2050.92	90.5495
7	1900.5	90.4072	8	1494.56	91.6448	9	1211.08	91.1576
10	827.312	86.3336	11	556.363	91.131	12	517.793	91.9724
13	478.26	95.2276	14	461.868	95.4274	15	428.12	92.2062
16	412.692	98.336						

Figure 4: FTIR graph for Thyroid sample



[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3837.65	97.0631	2	3743.15	95.706	3	3673.73	96.6563
4	3648.66	96.1913	5	3613.95	95.7501	6	3565.74	96.4703
7	3450.03	97.7386	8	3027.69	98.0124	9	2362.37	95.5689
10	2015.25	98.688	11	1986.32	98.3888	12	1957.39	98.252
13	1741.41	91.5905	14	1646.91	97.7053	15	1519.63	96.335
16	1455.99	98.1788	17	1367.28	95.3276	18	1214.93	95.274
19	1033.66	97.4603	20	998.946	97.7101	21	904.451	97.8719
22	692.32	96.9467	23	584.325	97.4657	24	559.255	98.0468
25	530.328	92.8073	26	503.33	86.5941	27	437.762	91.3096

Figure 5: FTIR graph for Thyroid sample

3. DATA INTERPRETATION

This study discusses the interpretation of elemental analysis of sweat samples obtained from two distinct groups – individuals diagnosed with hypothyroidism who take medication and healthy individuals as control samples. The interpretation focuses on the following elements - Calcium (Ca), Potassium(K), Phosphorus (P), Zinc (Zn), Iron (Fe), Sulpher (S), Aluminium, Copper (Cu), Manganese (Mn), Chromium (Cr). This analysis highlights the variations between the groups considering altered elemental levels. The descriptive statistical analysis of these elements was performed considering their mean, standard deviation, range, skewness, and kurtosis, etc. Among thyroid patients, potassium levels were considerably reduced (Mean = 10.53, SD = 2.46) compared to healthy individuals (Mean = 21.83, SD = 3.22). Similarly, zinc concentrations were also markedly lower in thyroid patients (Mean = 0.30, SD = 0.15) compared to healthy subjects (Mean = 0.83, SD = 0.23). On the contrary, phosphorus exhibited a higher mean in thyroid patients (Mean = 20.82) than in healthy ones (Mean = 15.10). Calcium, although elevated in thyroid samples, showed overlapping ranges between the thyroid and healthy individuals.

A two-way ANOVA was conducted to assess the effects of health status (healthy and thyroid individuals) on their variations in the elemental composition. The analysis gave a statistically significant result with a significance value of ($p = 0.011$), suggesting that there is a difference in the overall sweat composition between the two groups.

Post-hoc tests revealed that out of all the ten elements considered, potassium presented a marked reduction in thyroid patients, while the levels of phosphorus were slightly elevated. Phosphorus and Potassium show significant differences between the two conditions, with significance value ($p = 0.008$) and ($p < 0.001$), respectively. Despite the differences in the values of other elements such as calcium, copper, zinc, etc., the variations were not significant. This could be possible due to within-group variance. Other elements had no significant differences when compared. On considering the interaction effect between the conditions and elements, it indicated that the sensitivity of certain elements is higher compared to the rest of the elements with respect to the disorder condition, showing high significance ($p = < 0.001$).

Therefore, with 95% confidence, this study rejects the null hypothesis. The spectroscopic analysis on the sweat

samples was performed by using FT-IR to detect the presence of levothyroxine medication in sweat. Levothyroxine contains iodinated tyrosine residues, showing absorbance at 500-550nm range, due to halogenated aromatic compounds. The presence of C-I specific peaks in the range of 500–550 cm^{-1} aligns with the chemical structure of levothyroxine. On examining the sweat samples, all 20 samples, except sample 2 and 16 showed the presence of absorption peaks within or near 500–550 cm^{-1} confirming the presence of iodine-containing functional groups, which is consistent with levothyroxine. The variation in the peaks depicted the variability in the concentration levels of drugs in sweat. Some of the samples (samples 1, 3, 4, 5, 9, 11, 12, 14, 17, 18, 20), almost 50% of them, showed dual band patterns. These dual-band patterns enhance the confirmation of the levothyroxine in sweat. The presence of C–I stretch across all samples suggests a strong relation between levothyroxine medication and its excretion through sweat. Since iodine is not commonly found in sweat, it can be considered as a diagnostic marker for drug adherence.

Table 4: Descriptive Statistics

Elements	Condition	Frequency	%	Mean	Median	Mode	Std. Deviation	Variance	Min	Max	Range	Skew	Kurtosis	Mean ± Std.
Calcium	Thyroid	20	80%	42.28	42.62	35.01	3.44	11.81	35.01	50.05	15.04	-0.05	0.52	42.28 ± 3.44
	Healthy	5	20%	38.53	38.19	36.46	1.72	2.94	36.46	41.01	4.55	0.51	0.22	38.53 ± 1.72
Phosphorus	Thyroid	20	80%	21.27	20.54	15.39	3.82	14.6	15.39	30.52	15.13	0.56	0.03	21.27 ± 3.82
	Healthy	5	20%	15.5	15.95	10.49	4.21	17.73	10.49	21.2	10.72	0.21	-0.88	15.5 ± 4.21
Potassium	Thyroid	20	80%	9.8	8.78	2.26	5.39	29.07	2.26	22.77	20.51	0.93	0.66	9.8 ± 5.39
	Healthy	5	20%	21.42	20.77	11.52	6.93	48	11.52	28.96	17.44	-0.49	-0.52	21.42 ± 6.93
Manganese	Thyroid	20	80%	0.84	0.84	0.62	0.14	0.02	0.62	1.1	0.48	0.06	-1.03	0.84 ± 0.14
	Healthy	5	20%	0.91	0.84	0.74	0.21	0.04	0.74	1.22	0.48	1.69	3.17	0.91 ± 0.21
Copper	Thyroid	20	80%	14.9	14.92	10.44	2.26	5.1	10.44	18.57	8.13	-0.22	-0.51	14.9 ± 2.26
	Healthy	5	20%	13.88	14.78	9.86	2.91	8.47	9.86	17.11	7.25	-0.54	-1.17	13.88 ± 2.91
Aluminium	Thyroid	20	80%	0.92	0.9	0.72	0.16	0.02	0.72	1.42	0.7	1.96	5.42	0.92 ± 0.16
	Healthy	5	20%	1.3	1.12	0.86	0.56	0.31	0.86	1.93	1.06	1.32		1.3 ± 0.56
Sulpher	Thyroid	20	80%	8.79	8.52	5.89	1.82	3.31	5.89	12.47	6.58	0.31	-0.12	8.79 ± 1.82
	Healthy	5	20%	8.57	7.61	4.78	3.25	10.56	4.78	13.44	8.67	0.72	0.7	8.57 ± 3.25
Chromium	Thyroid	20	80%	0.74	0.77	0.59	0.09	0.01	0.59	0.92	0.34	-0.01	-0.54	0.74 ± 0.09
	Healthy	5	20%	0.81	0.68	0.57	0.32	0.1	0.57	1.17	0.6	1.52		0.81 ± 0.32
Zinc	Thyroid	20	80%	0.38	0.24	0.2	0.41	0.17	0.2	1.88	1.68	3.8	14.82	0.38 ± 0.41
	Healthy	5	20%	0.69	0.62	0.28	0.4	0.16	0.28	1.22	0.94	0.81	0.1	0.69 ± 0.4
Iron	Thyroid	20	80%	1.49	1.54	1.54	0.2	0.04	1.09	1.77	0.68	-0.76	-0.2	1.49 ± 0.2
	Healthy	5	20%	1.58	1.5	1.11	0.37	0.13	1.11	2.1	0.99	0.34	0.33	1.58 ± 0.37

Table 5: Two-Way ANOVA to assess the health status

	Type III Sum of Squares	df	Mean Squares	F	p	η2p

Condition	49.15	1	49.15	6.58	0.011	0.03
Calcium-Phosphorus-Potassium-Manganese-Copper-Aluminium-Sulpher-Chromium-Zinc-Iron	36901.85	9	4100.21	549.06	<.001	0.96
Condition x Calcium-Phosphorus-Potassium-Manganese-Copper-Aluminium-Sulpher-Chromium-Zinc-Iron	684.04	9	76	10.18	<.001	0.31
Error	1545.81	207	7.47			

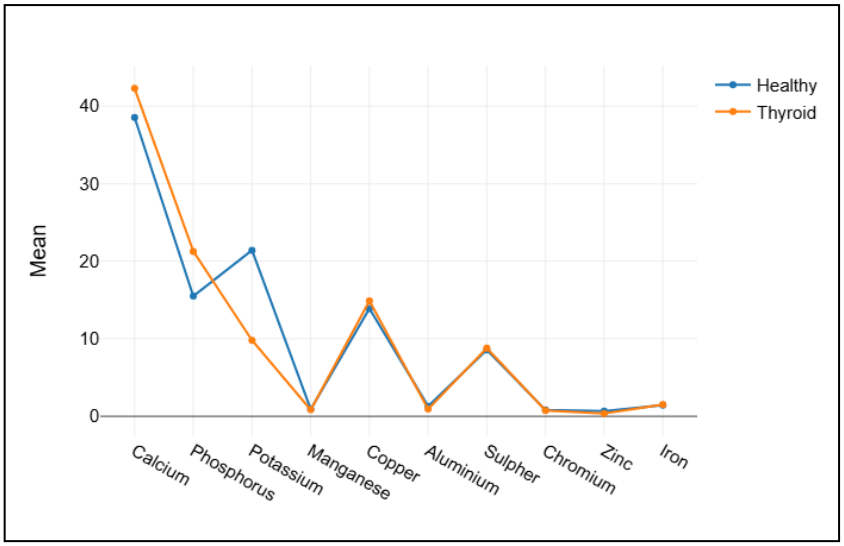


Figure 6: Line graph of elemental variation between Healthy and Thyroid individuals

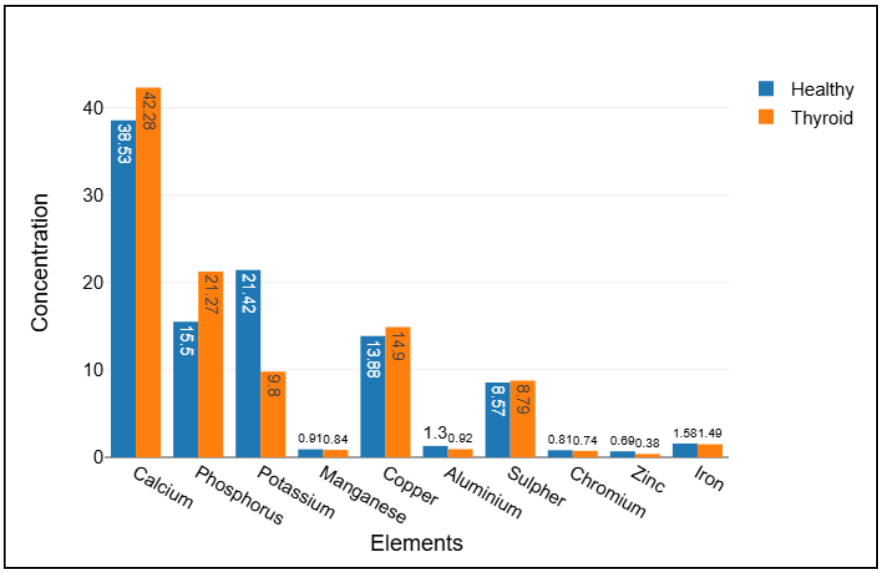


Figure 7: Bar graph of elemental concentration between Healthy and Thyroid individuals

4. DISCUSSION

This study aimed to investigate sweat samples from thyroid patients and healthy individuals using a dual-analytical approach: Energy Dispersive X-ray Fluorescence (EDXRF) for elemental profiling and Fourier Transform Infrared Spectroscopy (FTIR) for molecular drug detection. FT-IR analysis was done to detect the presence of levothyroxine in the sweat due to perspiration. It revealed the absorption peak in the range of 500–550 cm^{-1}, depicting the C-I bond, which is a characteristic component of levothyroxine, making it a reliable biomarker for detection. Except the samples 2 and 16, rest of them showed C-I peaks, detecting levothyroxine in sweat, with samples - 1, 3, 4, 5, 9, 11, 12, 14, 17, 18, 20 that showed dual band patterns signifying the presence of multiple vibrational states of the drug, compound with two distinct functional groups or its binding with sweat components in the matrix. The absence

of a dualband pattern does not imply that samples nullify the presence of levothyroxine, but it may suggest that the concentration of such samples could be low, or it could be due to variation in the metabolite interactions. EDXRF analysis involved the statistical analysis using a web tool Statisty, which included descriptive statistical analysis, two-way ANOVA without repeated measures, followed by Bonferroni post-hoc testing to detect any variations. The descriptive statistics supported the selective abnormalities of elements in thyroid dysfunction. While not all elements showed statistically significant differences when analyzed by condition alone, the condition × element interaction was highly significant, confirming that element-level changes are condition-specific and its not uniform across the elemental spectrum. On the overall analysis, the main effect of condition showed statistical significance ($p=0.011$) between the two groups. Further analysis by using a Bonferroni post-hoc test showed a significant difference between thyroid and healthy individuals only on two elements – potassium and phosphorus. Potassium levels were significantly reduced in thyroid patients, while phosphorus concentration was higher in thyroid patients. Although other elements such as calcium, zinc, copper, and iron showed variations, they were statistically insignificant ($p=>0.05$), suggesting that the variations could be normal biological variability.

5. LIMITATIONS AND CHALLENGES

Several factors complicate sweat-based analyses in this study. Hydration status, diet, ambient temperature, and individual metabolism all influence sweat composition, producing variability that makes molecular fingerprinting results inconsistent and complicates standardization. Sweat concentrations of levothyroxine and its metabolites may be much lower than in blood or urine, requiring highly sensitive detection techniques, and the complex mix of salts, proteins, and metabolites in sweat can interfere with FTIR spectra and EDXRF elemental analysis. The lack of general databases for drug-specific molecular fingerprints in sweat further hampers comparison and interpretation, highlighting the need for a consolidated pharmaceutical sweat database for forensic and clinical use. No standardized protocols, legal validations, or universally accepted threshold values exist for sweat-based drug detection, which limits forensic and medical interpretation. Methodological limitations in this study include a small, unbalanced sample (20 thyroid patients versus 5 healthy individuals), which reduces statistical power and increases bias risk, and sensitivity issues evidenced by two samples lacking clear FTIR C–I peaks, suggesting possible drug degradation or insufficient detection. Finally, despite non-normal distributions and unequal group sizes, parametric tests (ANOVA with Bonferroni post hoc) were used; these can show useful trends but have limited reliability under the present data conditions.

6. CONCLUSION

This study explored the biochemical and molecular profile of sweat in two groups of individuals- one group with people suffering from thyroid dysfunction and the other group of healthy individuals. By comparing the sweat samples from thyroid patients and healthy individuals identified condition-specific elemental variations and confirmed the excretion of levothyroxine, the most common thyroid medication prescribed for hypothyroidism. This study utilized non-invasive instruments such as EDXRF and FTIR to analyse the sweat composition of thyroid patients in comparison to healthy individuals, revealing the variations in the condition linked with elemental composition and confirming the presence of levothyroxine in sweat. Descriptive statistics were used first to identify elemental variability in sweat samples, which revealed marked deviations in potassium, phosphorus, calcium, and zinc in thyroid patients. These observations were verified by inferential statistics - ANOVA and post-hoc tests, which confirmed significant condition-element interactions, particularly for phosphorus and potassium. A strong interaction between the condition and specific elements showed statistical significance with reduced potassium levels and increased phosphorus levels in thyroid patients. These findings show that sweat can reveal specific biochemical changes that are linked to thyroid dysfunction, suggesting that the analysis of multiple elements at once is more useful in comparison with a single biomarker. FTIR analysis confirmed the levothyroxine excreted through sweat through a consistent C-I peak across all samples (500-550 cm^{-1}), with about 50% of the samples showing dual band peaks, indicating variations in the vibrational modes due to the interaction of the drug with the sweat matrix or compound with two distinct functional groups. The absence of the C-I peak in samples 2 and 16 could be due to various factors, such as the medicine could have completely metabolized, the concentration was below the FT-IR detection limit, or there could have been an interaction with the sweat matrix. EDXRF and FTIR are strong tools that can be used for medical as well as forensic testing in sweat analysis, providing a non-invasive approach to monitor both overall health and drug detection.

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