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GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC ANALYSIS OF VOLATILE CONSTITUENTS IN SALIVA

APRIL LOCHNER, SHARY WEISNER and ALBERT ZLATKIS*

Chemistry Department, University of Houston, University Park, Houston, TX 77004 (U.S.A.)

and

BRIAN S. MIDDLEDITCH

Department of Biochemical and Biophysical Sciences, University of Houston, University Park, Houston, TX 77004 (U.S.A.)

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SUMMARY

Present methods for the development of metabolic profiles are limited to the use of head-space techniques and solvent extraction methods. A new method for the development of saliva profiles which provides information complementary to existing analyses has been developed. The results of the developed methodology provide a reliable, reproducible method for metabolic profiling. Gas chromatographic—mass spectrometric analysis of the volatile constituents provided positive identification of 39 compounds. Application of the developed protocol toward the investigation of saliva as a vehicle for the non-invasive detection of certain pathological states, specifically diabetes mellitus and liver disorders, may be possible.

INTRODUCTION

The development of specific profiles has proven to be of analytical use in a number of areas of scientific investigation. The essence of profile analysis is the development of a highly characteristic fingerprint pattern which can be used either for identification or for correlation with qualitative and quantitative information.

In the biomedical field, the concept of metabolic profiling is increasingly

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recognized as an effective means of monitoring the highly integrated states of health and disease. Biological activity and cellular metabolism are highly ordered processes involving many enzymes, substrates, metabolites and reaction intermediates. By monitoring these substances, information regarding these intricate metabolic processes can be acquired. Similarly, an imbalance resulting from a dysfunction or a disease state may also be apparent. An advantage of using biologic, or metabolic, profiles is that they contain a great deal of quantitative and qualitative information reflecting the physiological state of an individual. As such, a comparison of the profiles obtained from normal and pathological states may provide important information as to the etiology, pathogenesis or diagnosis of certain diseases.

Much of the pioneering research on metabolic profiles and their relation to health and disease was performed by Zlatkis et al. [1], Liebich et al. [2], and Robinson and Pauling [3]. The results of these and other studies have provided insight into many human biochemical disorders. Notable among these are the diagnosis of diabetes mellitus by analysis of urinary volatiles [1], the discovery of inborn errors of metabolism [4] and the investigation of rheumatoid arthritis [5] and breast lesions [6].

Although there are a large number of biological fluids available for analysis, the fluids most commonly used for profiling are those which are present in sufficient quantities and relatively easy to obtain. These include blood, serum, cerebrospinal fluid, saliva, urine, perspiration and synovial fluid. Of the analytical techniques currently available for the profiling of biological fluids, high-resolution capillary gas chromatography (GC), combined gas chromatography—mass spectrometry (GC—MS) and high-performance liquid chromatography are the methods most widely used. These techniques provide the necessary degree of resolution combined with forms of detection which are broad-based and compatible with components present in trace amounts.

When considering the analysis of complex mixtures, the method of sample treatment and its compatibility with the chosen methods of separation and detection become important. Preparation methods for biological fluids compatible with GC have been classified into three general categories: direct chromatographic injection, headspace techniques and solvent extractions [7]. Each technique has both advantages and disadvantages, some methods being more appropriate for certain types of analyses.

Direct chromatographic injection involves the injection of untreated sample onto the chromatographic column. An advantage of this method is that it allows for quantitative analysis of highly volatile substances and substances not easily extractable. However, in general, the nature of biological fluids prevents the use of this technique since the presence of large amounts of inorganic salts and relatively high-molecular-weight biomolecules frequently destroy the analytical column. In addition, since there is no sample preconcentration step, there can be a problem with insufficient sensitivity for trace components [8].

Headspace techniques which include the selective trapping of organic volatiles onto adsorbent polymers are the most frequently used methods of sample preparation for biological fluids [7]. This technique has been widely used in the analysis of biological fluids and the development of profiles [1, 3, 5, 9—13].

Solvent extraction techniques represent the final category of sample pretreatment methods for biological fluids. In contrast to direct chromatographic injection and headspace techniques, extraction methods provide better representation of the less volatile, higher-molecular-weight components. Results from extraction techniques, therefore, are considered to be somewhat complementary to the other pretreatment methods [7].

It is the purpose of this research effort to develop a method for metabolic profiling of a biological fluid which examines a class of compounds not previously considered by other techniques. This includes components of low volatility and those requiring derivatization for analysis. In addition, this research effort will examine possible applications for the developed protocol, including the potential for the detection of ovulation in fertile women, the ability to diagnose certain disease states and the utility of a biological fluid for the determination of serum cholesterol levels.

Although any biological fluid can be used for the development of metabolic profiles, saliva, in some respects, represents an ideal body fluid for examination, and as such, was chosen for the present study. Saliva is known to contain a great deal of biochemical information, while its collection is non-invasive.

In contrast to well studied serum, the composition of saliva has only become a matter of detailed investigation in the last few decades. The results of these studies make it increasingly clear that saliva is a biological fluid of rare quality. Indeed, it may be appropriate to think of saliva as an ultrafiltrate of blood, reflecting much of the same biochemical and metabolic information, yet retaining certain unique characteristics of its own.

Inherent to the nature of profile analysis is the complex nature of the sample of interest. Such is the case with saliva. Saliva is known to contain a wide variety of biologically significant compounds and has been analyzed with regard to fatty acids and lipids [14], amino acids [15], steroids [16], glycoproteins [17], carbohydrates [18], organic acids [19] and volatiles [20]. These compounds are known to be present over a wide dynamic range of concentrations, many of which exist at trace levels [8]. Not only is saliva a complex medium, it is also dynamic in nature with a number of factors governing the amount and type of constituents present at any given time. Although previous research has largely focused on specific classes of compounds, it is apparent that saliva is ideally suited for profile analysis.

This work describes the development of a new method for the metabolic profiling of saliva which is complementary to previous profiling techniques.

EXPERIMENTAL

Analysis of extractable constituents

A procedure was devised which would allow for the examination of the extractable constituents of saliva. For any extraction process involving aqueous media, the extracting solvent ideally must display several characteristics; high purity, low water solubility, high volatility and high solvating power for the components of interest. High volatility of the extracting solvent is particularly desirable for compounds which require concentration prior to analysis. The higher the volatility, the more easily the solvent can be removed with minimal loss of the more volatile extracted constituents.

Of the various solvents evaluated, methyl *tert.*-butyl ether (MTBE) possessed all of the above characteristics and appeared to extract the greatest variety of constituents from saliva. MTBE is a solvent which is manufactured in sufficiently high purity for trace analysis, extracts both polar and non-polar substances from aqueous media and is less flammable and less prone to form explosive peroxides than diethyl ether.

Preliminary identities of the major constituents of these extracts were then determined by combined GC—MS using a Finnigan 1020/OWA instrument. The major constituents were squalene and cholesterol (both characteristic of human skin) and a series of partially resolved components tentatively characterized as wax esters (esters of fatty acids and fatty alcohols). It was determined that there might be some difficulty in discerning quantitative variations in the wax ester region of the chromatogram since there were so many partially resolved components. To overcome this, the saliva was saponified prior to extraction so that the putative wax esters would be cleaved to obtain fatty acids and fatty alcohols. Saponification was accomplished by heating with sodium hydroxide for 20 min. The saponified mixture was acidified and extracted with MTBE prior to analysis. Thus, the neutral and acidic products were analyzed, while the basic constituents remained in the aqueous medium after extraction. Analysis of basic components revealed few components of interest, and for this reason, attention was focused on the acidic and neutral fractions for profile development.

Analytical protocol

The selected method for screening was specifically designed for the trace analysis of extracted, derivatized components in saliva. The sampling materials and methods are outlined below.

Reagents and columns

The saliva samples were saponified with a 5.0 *M* solution of sodium hydroxide. The solution was prepared by weighing 20.0 g of sodium hydroxide pellets obtained from American Scientific Products (Mallinckrodt, reagent grade) and adding 100.0 ml of water obtained from the Milli-Q water purification system by Millipore. The sodium hydroxide solution was stored in a polyethylene bottle.

The saponified samples were acidified with concentrated hydrochloric acid.

Saliva samples were extracted with MTBE purchased from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). The solvent was suitable for pesticide analysis, spectrophotometry, liquid chromatography and GC.

The extracted components were derivatized prior to analysis with bis(trimethylsilyl)trifluoroacetamide (BSTFA) purchased from Supelco (Bellefonte, PA, U.S.A.) in 100-μl ampuls.

All reagents were chromatographed where possible to determine the levels of impurities and any possible interference with sample components.

Initial work was performed on 35 m $\times$ 0.32 mm I.D. fused-silica WCOT column coated with DB-5 (5% phenylmethylphenylsilicone), 0.5 μm thickness, purchased from J & W Scientific (Rancho Cordova, CA, U.S.A.). Later work was performed on a 50 m $\times$ 0.22 mm I.D. fused-silica WCOT column coated

with CP sil 8 CB (8% phenylmethylphenylsilicone), 0.13 μm thickness, purchased from Chrompack (Bridgewater, NJ, U.S.A.). The upper temperature limit of these columns is 300°C for isothermal separations or 325°C for temperature-programmed separations.

Saliva samples

The saliva samples used for the development of the analytical protocol were donated from volunteers associated with our laboratory. All subjects were instructed to thoroughly rinse their mouths prior to sample collection. Volumes between 1 and 50 ml of whole saliva were collected in a clean glass container and analyzed immediately or frozen for analysis at a later time.

Sample preparation

Saliva samples were collected and examined according to the following protocol. The developed procedure involves a fifty-fold concentration factor for the extracted components. (1) Transfer 1.0 ml of saliva into a precleaned glass centrifuge tube with a PTFE-lined screw cap. (2) Heat with 10 μl of 5 *M* sodium hydroxide solution at 100°C for 20 min. (3) Cool the tube and acidify the contents with 5 μl of concentrated hydrochloric acid. Vortex the tube. (4) Extract with 1.0 ml of MTBE. Vortex for 1 min, then centrifuge for 10—15 min (9750 *g*) to separate the aqueous and organic layers. (5) Transfer the upper (organic) layer to a 1-ml tapered vial and evaporate the solvent under a stream of dry nitrogen. (6) Add 20 μl of BSTFA and heat at 60°C for 15—20 min, rotating and wetting the walls of the vial periodically. (7) Use 1.0–3.0 μl for the GC analysis.

Gas chromatographic conditions

Column 1: 35 m × 0.32 mm I.D.; DB-5, 0.5 μm thick. Operating conditions: detector, flame ionization; injection port temperature, 200°C; detector temperature, 350°C; carrier gas (helium) flow-rate, 6 ml/min; split ratio, 3:1. Chromatographic program: initial temperature, 150°C; initial time, 4 min; program-rate, 6°C/min; final temperature, 300°C; final time, 10—20 min; time of analysis, 40—45 min. Column 2: 50 m × 0.22 mm I.D.; CP sil 8 CB, 0.13 μm thick. Operating conditions: detector, flame ionization; injection port temperature, 200°C; detector temperature, 350°C; carrier gas (helium) flow-rate, 2 ml/min; split ratio, 3:1. Chromatographic program: initial temperature, 150°C; initial time, 0 min; program-rate, 4°C/min; final temperature, 300°C; final time, 13—25 min; time of analysis, 45—50 min.

Mass spectrometric conditions

MS analyses were conducted using the Finnigan 1020/OWA and the Hewlett-Packard 5987A mass spectrometers. The Finnigan instrument contained the NBS library of approximately 38 000 compounds, the Hewlett-Packard instrument was equipped with the NBS, Cornell, Wiley and MSU Biomedical Libraries totalling approximately 78 000 compounds.

The Finnigan 1020/OWA quadrupole mass spectrometer has a direct GC—MS interface and employed a highly efficient oil diffusion pump at the ion source housing. The experimental conditions were as follows: ionization voltage 70

eV, ion source temperature 200°C, operating pressure $1 \cdot 10^{-5}$ Torr. The reconstructed-ion chromatogram from a second ion source with an ionization voltage of 20 eV was used as the signal for the GC trace. Mass spectra were recorded in the mass range *m/e* 50—600 at a scan-rate of 500 u/s applying the mode of automatic repetitive scanning. This instrument can only be operated in the electron-impact ionization mode; maximum resolution is 1600 u, and the mass range is 4—800 u.

The Hewlett-Packard 5987A quadrupole mass spectrometer was equipped with a direct GC—MS interface and a differentially pumped vacuum system. The experimental conditions were as follows: ionization voltage 70 eV, ion source temperature 220°C, operating pressure $1 \cdot 10^{-6}$ Torr. Mass spectra were recorded in the range *m/e* 50—800 at a scan-rate of 2000 u/s applying the mode of automatic repetitive scanning. This instrument can be operated in a variety of ionization modes; electron impact was chosen for this analysis. The mass accuracy is to +0.12 μm within the calibrated mass range which extends from 10 to 2000 μm.

Because of the lower sensitivity of a mass spectrometer over a flame detector, qualitative analyses were conducted using a 50-ml saliva sample. The

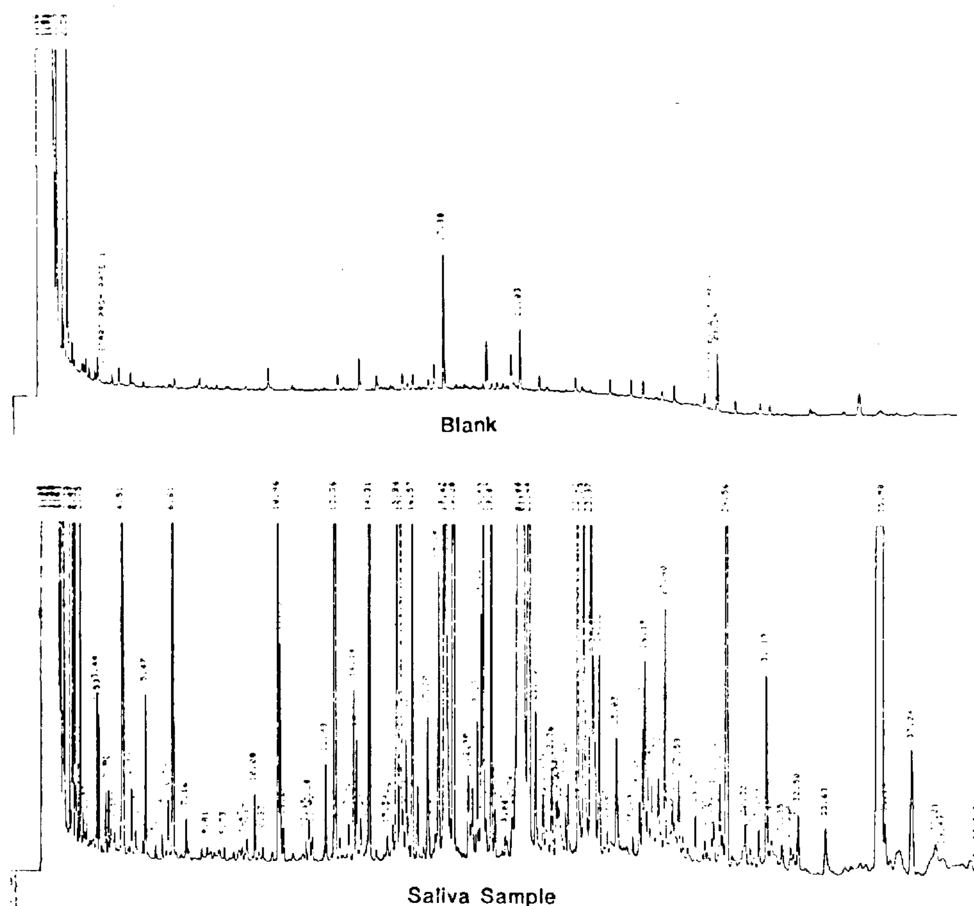

Fig. 1. Profiles obtained for a saliva sample and an accompanying blank.

saponification and derivatization procedures were scaled up accordingly. Both mass spectrometers were equipped with a DB-5 column and operated under similar chromatographic conditions. Spectra were recorded and stored on magnetic tape with both instruments. Substances were identified by comparison with spectra of reference compounds within the data base systems.

Analysis of saliva according to this analytical protocol resulted in reproducible chromatograms with numerous peaks sufficiently well resolved for easy qualitative and quantitative comparisons.

RESULTS AND DISCUSSION

The results of the present study indicate that the developed procedure does provide an effective, reproducible means of obtaining individual metabolic profiles from saliva. Typical chromatograms have been included to provide an indication of the type of data produced with the final analytical protocol.

Fig. 1 shows a profile obtained from a saliva sample and an accompanying blank. The blank analysis was a total system blank: the entire analytical

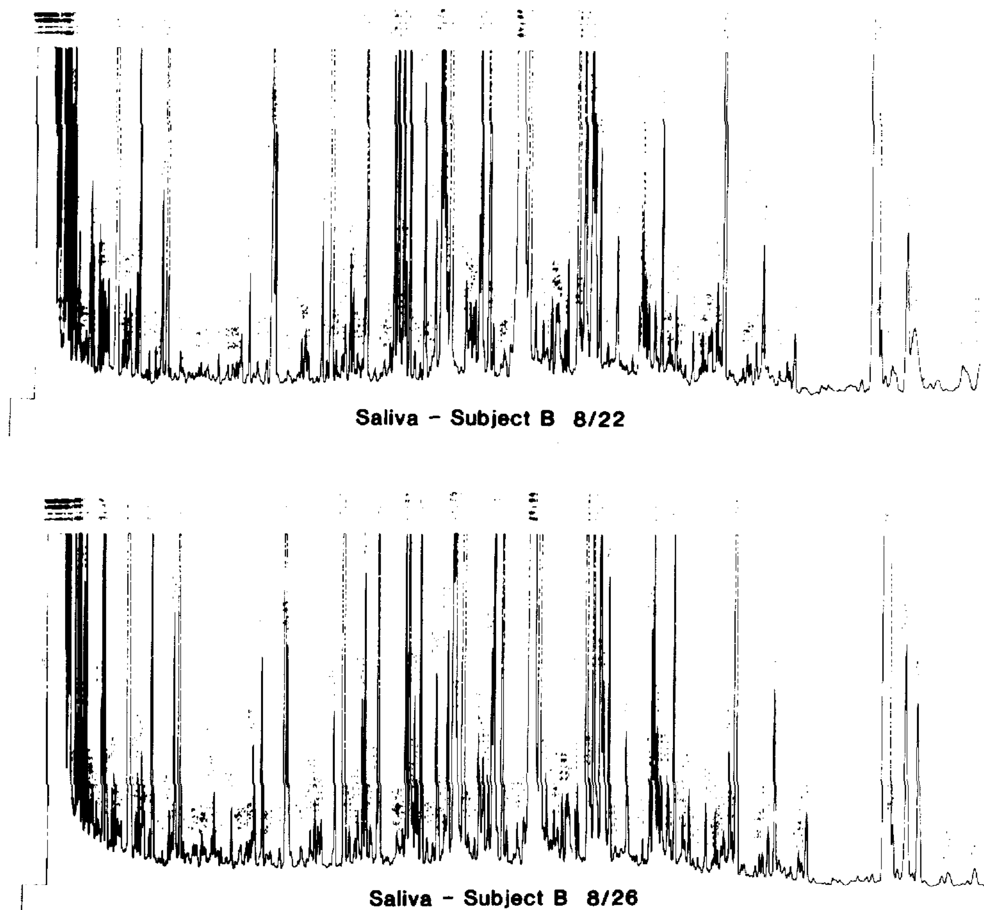

Fig. 2. Profiles obtained for saliva from subject B on two different days demonstrating good reproducibility.

procedure was adhered to, with 1 ml of Milli-Q water being substituted for the saliva sample. A total system blank with the saliva omitted and no Milli-Q water substituted gave similar results. As indicated, there is no appreciable contribution from the blank to the actual sample.

Fig. 2 shows the degree of reproducibility obtained from saliva samples collected four days apart from the same individual. The trimethylsilyl ether of cholesterol has highly reproducible retention times of 35.74 and 35.76 min, respectively, in these chromatograms. (Differences in retention times for these and other chromatograms may be explained on the basis of slight variations in carrier gas flow-rates.) Note the close similarity in the overall appearance between the two chromatograms. Differences which do exist are mostly quantitative in nature and quite small. The only major qualitative difference is the appearance of a peak with retention time 36.46 min in the lower chromatogram. Periodic examination of saliva by this technique over a period of one month from the same individual indicates a remarkable consistency in the profiles.

Fig. 3 shows the degree of reproducibility in the saliva profiles of two individuals of opposite sex. Although there is close similarity between the two chromatograms, there are both qualitative and quantitative differences. The

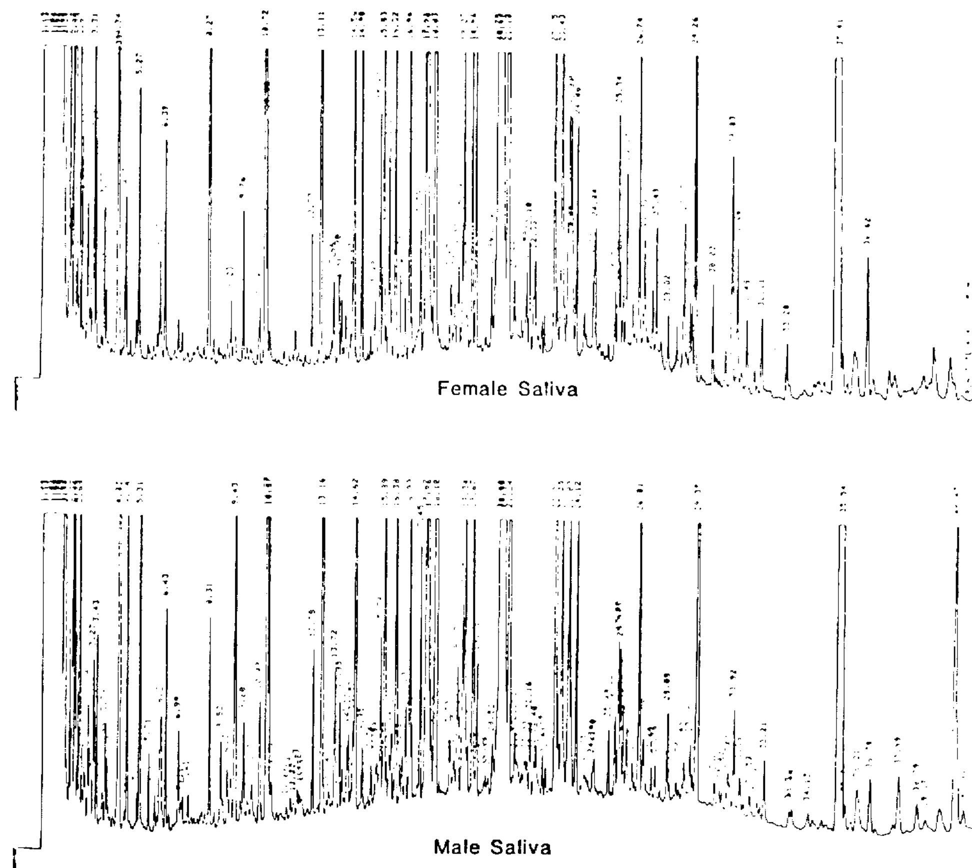

Fig. 3. Profiles obtained for saliva from female and male subjects.

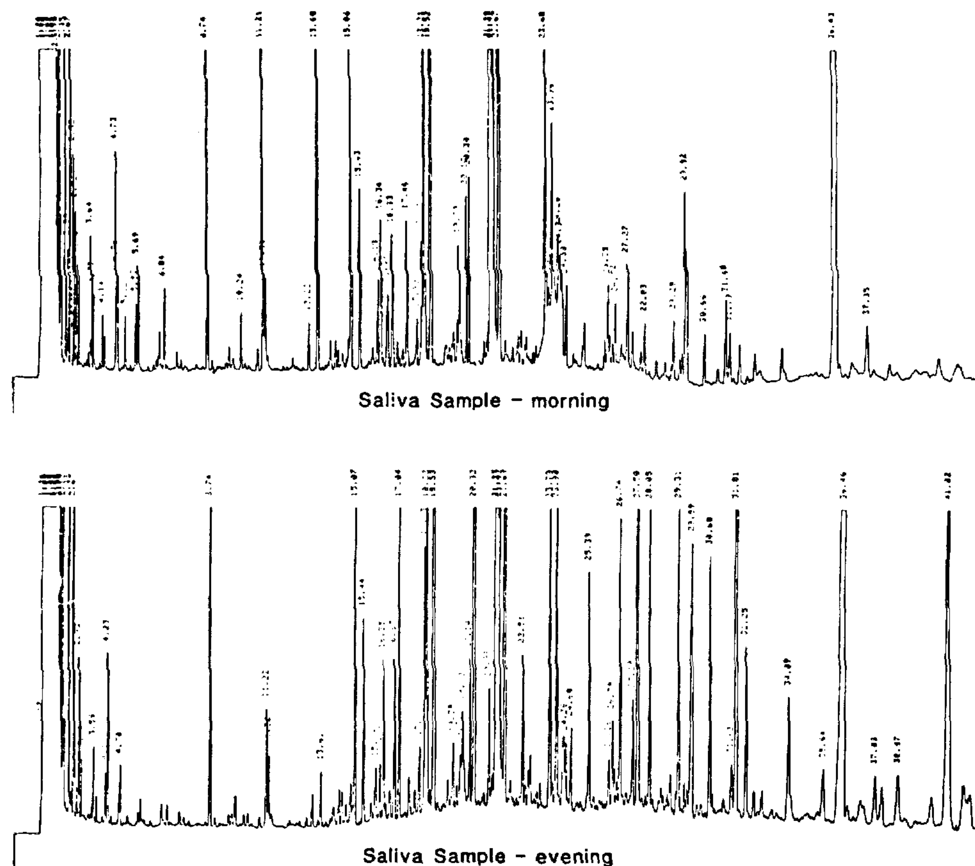

Fig. 4. Profiles obtained for saliva from the same individual collected 12 h apart. Note the greater abundance of later eluting peaks in the evening profile.

chromatogram of the female saliva shows a significant peak eluting at 14.98 min which is greatly reduced in the male saliva. The male saliva profile indicates the presence of two compounds eluting at 9.43 and 40.47 min which are absent in the female saliva profile.

Fig. 4 shows a comparison of chromatograms obtained from saliva samples collected from the same individual separated by a period of 12 h. The evening sample demonstrates a greater abundance of heavier, less volatile components relative to the morning sample. The most obvious differences is the presence of the peak at 41.02 min in the chromatogram of the evening sample.

Studies indicate that the profile obtained by periodic sampling of a large volume of saliva does not change significantly with time if the saliva is sealed and refrigerated. Similarly, a sample, once prepared, does not change over a period of several months if refrigerated.

Qualitative analysis by mass spectrometry

Analyses were conducted using a Finnigan 1020/OWA and a Hewlett-Packard 5987A mass spectrometer. Both instruments were equipped with a DB-5 column and operated under similar chromatographic conditions. The combined

results of these analyses led to the positive identification of 37 compounds with a high degree of certainty. These are listed in Table I. With the exception of phenol, none of these compounds have been identified by researchers using other profiling techniques on saliva [21], establishing the devised protocol as a complementary method for profile analysis.

Compounds listed in Table I include eleven fatty acids: straight-chain and branched-chain, saturated and unsaturated. The presence of fatty acids as major components is to be expected since the sample work-up involves saponification of lipids. There is nothing unusual about the fatty acid composition of the saliva samples examined and these results are consistent with those of other investigators [14, 22].

Eicosanol and octadecanol are expected saponification products of wax esters; long-chain alkanes are known to be normal constituents of saliva.

4-Hydroxy-3-methoxymandelic acid (sometimes referred to as vanilline-mandelic acid or vanillylmandelic acid) is a common metabolite of catecholamines such as adrenaline and noradrenaline.

Decaethylcyclopentasiloxane and hexamethyldisiloxane are ubiquitous silicone compounds. Bis(2-ethylhexyl) phthalate and the other phthalate compounds are common plasticizers used in the U.S.A. and were present at a lower levels in the blank.

Squalene is characteristic of human skin and is an intermediate in the biosynthesis of cholesterol.

TABLE I

COMPOUNDS POSITIVELY IDENTIFIED BY COMBINED GAS CHROMATOGRAPHY—MASS SPECTROMETRY

Fatty acids	*Other compounds*
Caprylic acid ($C_{8:0}$)	*p*-Hydroxyhydrocinnamic acid
Lauric acid ($C_{12:0}$)	Phenylacetic acid
Myristic acid ($C_{14:0}$)	Phenol
Branched pentadecanoic acid ($C_{15:0}$)	2-Butanol
n-Pentadecanoic acid ($C_{15:0}$)	*n*-Octadecanol
Palmitoleic acid ($C_{16:1}$)	*n*-Eicosanol
Palmitic acid ($C_{16:0}$)	4-Hydroxy-3-methoxymandelic acid
Margaric acid ($C_{17:0}$)	Decamethylcyclopentasiloxane
Linoleic acid ($C_{18:2}$)	Hexamethyldisiloxane
Oleic acid ($C_{18:1}$)	Bis(2-ethylhexyl) phthalate
Oleic acid isomer ($C_{18:1}$)	Diethyl phthalate
Stearic acid ($C_{18:0}$)	Dibutyl phthalate
Arachidonic acid ($C_{20:4}$)	Dimethyl phthalate
Ricinoleic acid	Squalene
	Cholesterol
Alkanes	Cholesta-3,5-dien-7-one
Branched decane	
Tricosane	
Tetracosane	
Hexacosane	
Heptacosane	
Octacosane	
Nonacosane	

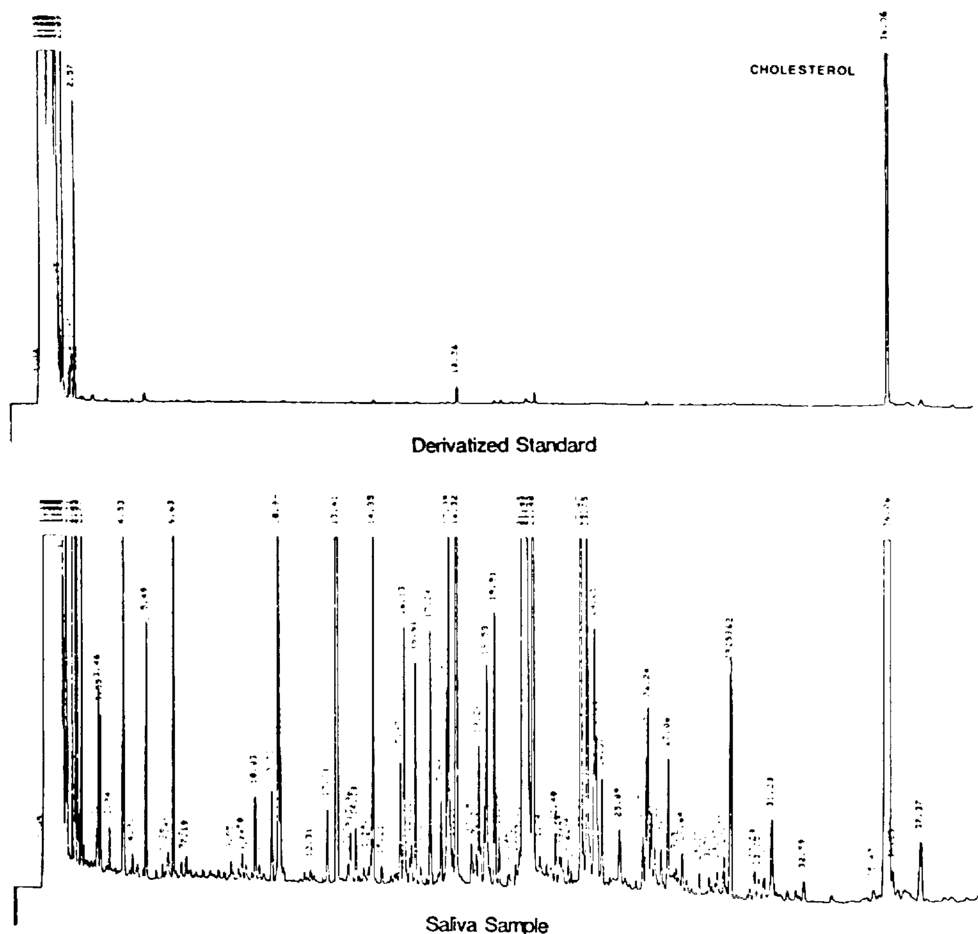

Fig. 5. Chromatogram obtained for BSTFA-derivatized external standard of cholesterol and a saliva profile. Note the identical retention times of 36.06 min for the trimethylsilyl ether of cholesterol in each chromatogram.

Cholesterol is the major blood sterol and cholesta-3,5-dien-7-one is an oxidation product of cholesterol.

Fig. 5 shows the chromatograms of derivatized cholesterol and a saliva profile. The trimethylsilyl ether of cholesterol has identical retention times of 36.06 min in each chromatogram. This, along with the mass spectral information, confirms the identity of this large late-eluting peak.

Applications

The analyses of biological fluids, for purposes of normal profiling and for disease detection, as well as for analyses of specific compounds, are areas of great potential. Saliva contents have been examined for changes which occur and possible diagnostic potential in dental diseases [23] and tuberculosis [24]. There is a growing record of saliva use by analytical biochemists for the determination of drug levels and studying pharmacokinetic metabolism [25, 26]. The particular advantage of using saliva in these applications is that saliva has

been shown to contain a great deal of biological and biochemical information, and it represents a medium for analysis which can be obtained from individuals in a non-invasive manner.

The protocol developed for metabolic profiling in the present study has application in a number of areas of research. The highly characteristic nature of the profiles makes them an effective means for comparing normal and abnormal states of health. Some potential applications of this analytical method are described below. An advantage of using this protocol in the following studies is that the technique affords a large amount of data for a broad range of chemical classes. The probability of finding specific indicators for these and other applications, therefore, is much greater than using techniques which focus on specific classes of compounds.

Detection of ovulation

Several investigators have reported changes in certain saliva constituents which can be shown to have some correlation with the menstrual cycle. Levels of progesterone [27] and dodecanol [28] in saliva have been correlated with

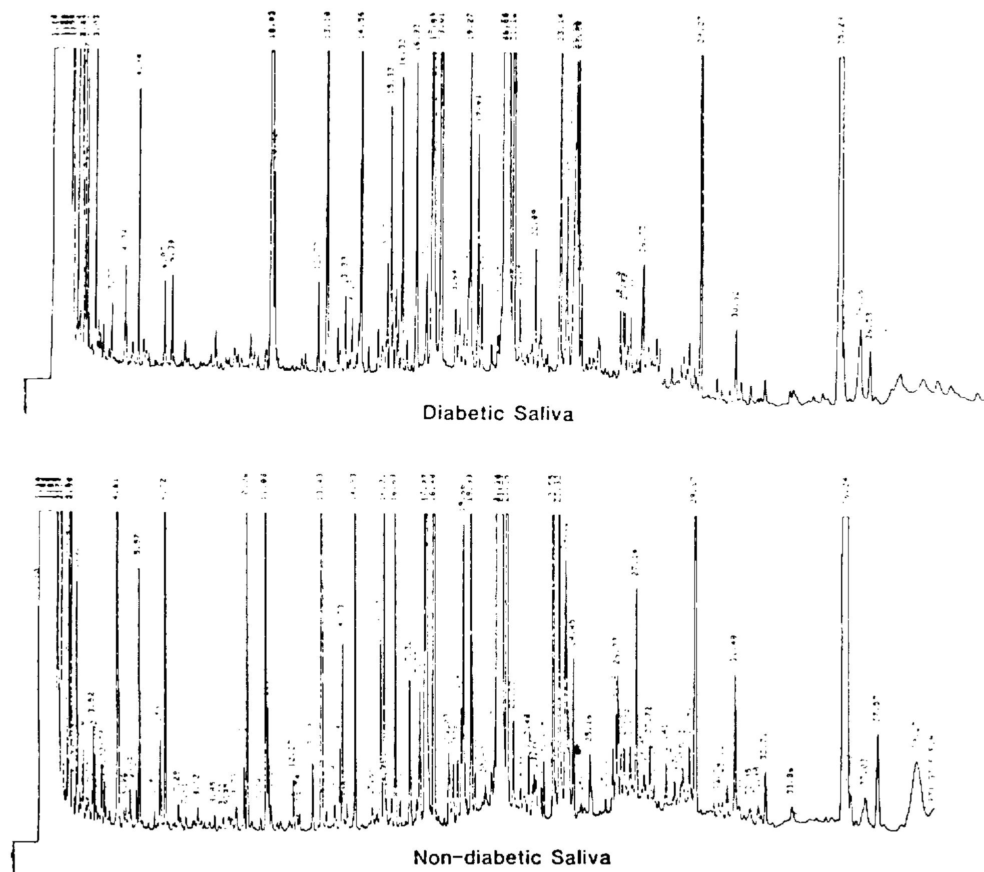

Fig. 6. Profiles obtained for saliva from diabetic (D8) and non-diabetic (N2) individuals. The diagnostic peak has a retention time of 26.29 min in the diabetic profile.

ovulation. Changes in several other components of saliva have been correlated with either basal body temperature shift or the midpoint of the cycle, but not with hormonal indicators of ovulation [29].

Diabetes mellitus

Since researchers have indicated that diabetes [1, 30] and other diseases [31- 33] could be diagnosed on the basis of differences in metabolic profiles, a pilot study was undertaken to see if there were any differences in saliva profiles which might also be of diagnostic or pathologic significance. A sample pool of ten individuals with documented diabetes mellitus was compared to seven individuals without any indication of overt diabetes. The details regarding sample analysis are shown in Table II.

Fig. 6 shows a comparison of saliva profiles obtained from diabetic (D8) and non-diabetic (N2) individuals. Visual comparison of all results revealed that the profiles from some of the diabetics exhibit a relatively strong, characteristic peak in the central region of the chromatogram.

TABLE II

DETAILS OF SAMPLES COLLECTED FROM PATIENTS WITH DIABETES MELLITUS AND LIVER DISORDERS

Subject	Day prepared	Day analyzed	Comments
Diabetes mellitus			
D1	January 23	February 4	
D2	January 23	January 31	
D3	January 23	February 4	
D4	January 23	January 31	0.25 ml Saliva
D5	January 23	February 5	
D6	January 23	February 4	
D7	January 23	February 5	
D8	January 23	February 4	
D9	January 23	February 4	
D10	January 23	February 4	
Liver disorder			
L1	January 23	February 4	
L2	January 23	February 4	
L3	January 23	January 31	
L4	January 23	February 5	
L5	January 23	January 31	
L6	January 23	February 5	
L7	January 23	February 5	
L8	January 23	February 4	
Normal controls			
N1	January 23	January 31	
N2	November 8	January 31	Subject C
N3	January 15	February 6	Subject E
N4	September 28	February 6	Subject X
N5	September 12	February 6	Subject B
N6	September 28	February 6	Subject D
N7	September 10	February 6	Subject A

Liver disorders

A similar comparison was made involving subjects with liver disorders to see whether any differences in their saliva profiles might prove to be of diagnostic value. The biological profiles of the saliva from eight individuals with documented liver disorders were compared to seven control individuals. The details of sample analysis are listed in Table II.

Fig. 7 shows a comparison of the saliva profiles obtained from a subject with a liver disorder (L4) and a normal control subject (N2). Examination of all profiles revealed that a few of the profiles from patients with liver disorders exhibit a relatively strong, characteristic peak early in the chromatogram. This peak has a retention time of 12.02 min in the profile for subject L4.

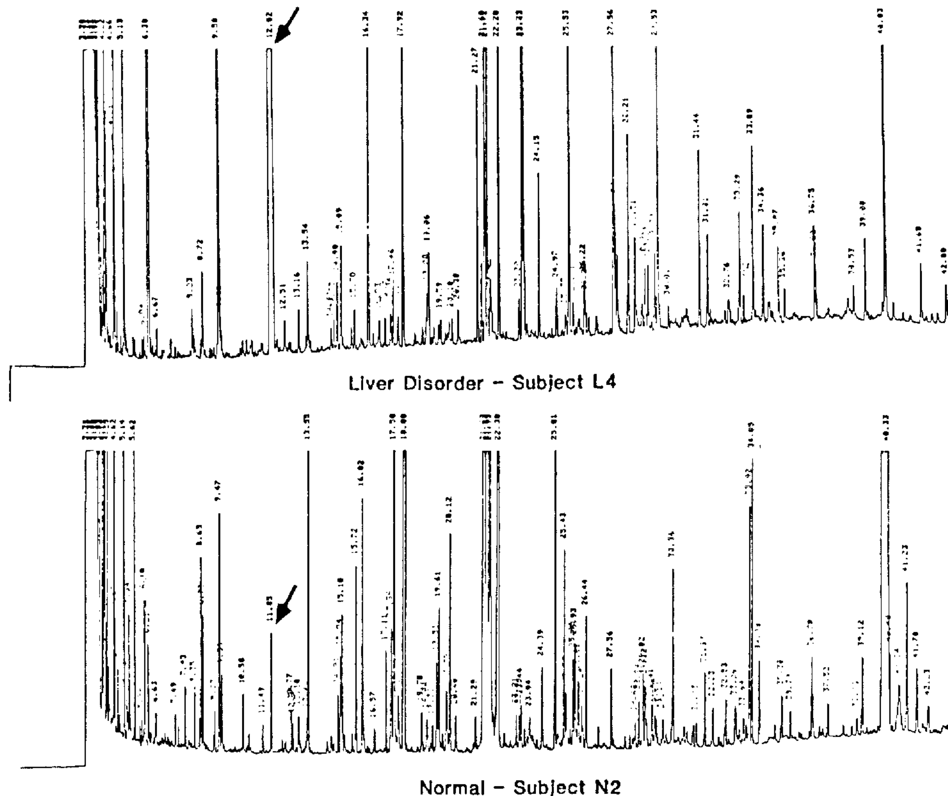

Fig. 7. Profiles obtained for saliva from a subject with a liver disorder (L4) and a normal individual (N2). The diagnostic peak has a retention time of 12.02 min in the liver disorder profile (arrow).

Cholesterol determinations

Common to all individuals tested was the presence of a significant cholesterol peak in the saliva profile. The percentage of the trimethylsilyl ether of cholesterol relative to total peak areas other than the solvent peak (first 4 min of the chromatogram) varied from 17 to 48%, depending in part on the individual being analyzed. Although differences in individuals were discernible, the percentage of cholesterol in the profiles from one subject demonstrated a substantial degree of consistency when analyzed over a period of time.

Research has shown saliva levels of cortisone and cortisol to be an accurate reflection of active blood levels [30], and strong serum-saliva correlations exist for other components [34]. The possibility exists, therefore, that saliva cholesterol levels might be related to blood cholesterol levels as well. If such an association exists, there is the potential for using saliva as a non-invasive means by which serum cholesterol can be monitored.

CONCLUSIONS

The specific goal of this research has been the development of a reproducible analytical protocol for the metabolic profiling of saliva. Analytical techniques were utilized which allowed for the discovery and characterization of extracted, derivatized constituents of saliva. A final method was established which afforded large amounts of data for a broad range of chemical classes not previously analyzed by other profiling techniques. The method developed provides chromatograms which contain an abundance of peaks sufficiently well resolved that they can be readily quantitated.

Consistent with expectations from a broad-based approach, separation of saliva components results in complex chromatograms with over 150 peaks for each sample. Combination of this chromatographic procedure with MS has permitted the positive identification of 37 components with a high degree of certainty. Two additional components were identified through retention time matching with external standards.

The developed procedure shows a high degree of reproducibility both for the same individual and for different individuals. The analytical protocol was utilized in some potential applications for metabolic profiling.

The results of the diabetes mellitus pilot study found significant differences in the metabolic profiles from diabetic and non-diabetic individuals. Less definitive results were found in the liver disorder study. A component in the profiles which may prove to be characteristic of a specific type of liver disorder was identified. These conclusions were based on visual examination of the profiles obtained from several individuals with each pathological condition and comparing them with the profiles obtained from control individuals. Specific peak area percentages were plotted and analyzed for differences which may reflect the presence, or absence, of a certain pathological state.

The presence of a significant cholesterol peak in all samples has suggested the possibility of a close serum—saliva cholesterol correlation. If this exists, saliva analyzed by the developed analytical protocol may provide a non-invasive means of monitoring serum cholesterol levels.

In conclusion, a reliable, reproducible method for the analysis of the extracted and derivatized volatile components of saliva has been developed which has application in a number of areas of research. In particular, it shows promise in the determination of certain disease states as well as providing a non-invasive means for monitoring serum cholesterol levels.

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