

Clinical Analysis of Sebum by Mass Spectrometry-A Brief Update

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The mammalian integument contains sebaceous-type exocrine glands throughout, excluding the palms and soles of feet. These glands secrete a lipid rich substance, sebum, across the integumentary surface. Sebum provides an antimicrobial film on the skin protecting against microbes [1], reviewed by ref. [2], its waxes help protect from dehydration in conjunction with stratum corneum lipids [3], provides photoprotection, is involved in antioxidant transport, wound healing, and absorption of topical drugs and analgesia. Sebum is a complex mixture of lipids including Free Fatty Acids (FFAs), triacylglycerides (TAGs) and other glycerolipids, squalene, sterols and their esters, and wax esters along with minor ceramide and glycerophospholipid components. Composition of broad lipid class ratio, degree of lipid saturation, and C chain length of lipid compounds can vary dramatically between body site and among species. Difference in sebaceous lipid physical and chemical properties can have dramatic influences on its function.

Cutaneous microbial infections and pilosebaceous diseases can alter sebum chemical composition and affect its protective function [4]. Sebum is composed primarily of TAGs and wax esters. Upon secretion microbial lipases hydrolyzes ester bonds to release smaller glycerolipids and FFAs. *Propionibacterium acnes* are a gram-positive bacterium that may cause acne, blepharitis, folliculitis, and endophthalmitis [5]. *P. acnes* grows within the anaerobic environment of pilosebaceous units and may induce inflammatory mediators. In humans, antimicrobial lipids including lauric acid (12:0), palmitic acid (16:0), oleic acid (18:1), and sapienic acid (16:1) have been shown to limit *P. acnes* growth [5]. *Malassezia restricta* and *M. globosa* consume saturated FFAs in sebum and can cause dandruff and seborrheic dermatitis [6]. *Malassezia* populations increase in humans when sebaceous activity is upregulated during puberty. Lipases degrade TAGs and *Malassezia* preferentially consumes saturated FFAs, leaving higher proportions of unsaturated FFAs. While the above two diseases usually do not have drastic medical consequences, they may lead to psychological problems such as perception of self-esteem or depression. While host microbiota may alter sebum chemical composition, other clinical applications can be deduced from sebum, such as alcohol dependence [7], photo-oxidative stress [8], and steroid deprivation [9]. Due to the complexity of sebaceous chemical composition, simple clinical applications to determine changes during cutaneous microbial diseases are desired.

Before sebaceous chemical composition can be determined, sebum must be collected, dissolved into a solvent, and concentrated to a desirable level. Several methods have been described for sebum isolation including inverting a flask with an appropriate solvent onto the integument several times, rubbing the skin surface with cotton balls wetted in 3:2 chloroform:methanol [10], applying bentonite clay on the skin [11], or using an absorbent paper [12]. Tape specially designed for the collection of sebaceous lipids is available under the name Sebutape[®] Adhesive Patches (cat# S100) or Sebutape[®] Indicator Strips (cat# S232) (CuDerm Corporation, Dallas, TX). While indicator strips are useful for determining sebaceous gland density, adhesive patches remove higher amounts of lipid in a shorter application time and are preferred for downstream analysis (personal observation). When sebum has been collected onto a sorbent material it can be dissolved in a mixture of chloroform/methanol and evaporated under a stream of N₂ for further analysis.

One of the most widely used methods for broad lipid class ratio determination is thin-layer chromatography (TLC) [13]. TLC has the advantages of being cheap, as an entire system can be purchased with an investment of a few hundred US dollars, and requires minimal technical expertise to collect results. When combined with scanning densitometry, quantitative ratios of broad lipid classes between samples can be obtained. In order to obtain reproducible results it is imperative to equilibrate chambers with solvent overnight, apply silicon grease between the chamber and the lid to create an airtight seal, place filter paper in the chamber, and keeping chambers clean with fresh solvent. Solvents used for separation are too numerous to list, but common solvents include hexane/ethyl ether/acetic acid to separate neutral lipids, isooctane/ethyl ether for nonpolar lipids, and chloroform/methanol/water for polar lipids. Additionally, multiple solvents can be used for a more complete separation. For detection of lipid classes after separation a common method is charring, in which the plates are sprayed with phosphoric acid with copper sulfate and heated until the lipid molecules appear black. However, if further downstream analyses, such as Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) MS is to be used, the lipids should be imaged with rhodamine 6G and visualized under a UV light.

The main downside of TLC is lack of information on individual lipid molecules. The more 'classical' approach to determining quantitative ratios of individual molecules is gas chromatography (GC)/MS. GC/MS is still widely used but has been replaced in many labs by liquid chromatography such as High Performance Liquid Chromatography (HPLC) coupled with electrospray ionization (ESI) tandem MS, which is not covered in this brief review (Table 1). To make lipids more volatile they can be hydrolyzed into Fatty Acid Methyl Esters (FAMES) by addition of a strong base or acid, depending on which method is used, and purified by liquid-liquid extraction with hexane. The advantage of FAME analysis is that they can be reacted and recovered within one hour in a single vessel. The use of hexane for purification also aids maintaining a clean instrument as it will not absorb water like alcohols and is not as irritating and corrosive as pyridine. The disadvantage of FAME analysis is the inability to determine FAMES from different lipid classes unless separation is performed a priori by TLC or solid phase extraction, which can add considerable time and expense to analysis. Derivation of total lipid extracts to trimethylsilyl (TMS) esters can be performed with hexamethyldisilazane/trimethylchlorosilane/pyridine 3:1:9 and quantified by high temperature GC/MS. This procedure can determine nearly all lipids in a total sebum extract in a single run;

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Received January 20, 2014; Accepted February 08, 2014; Published February 12, 2014

Citation: Pannkuk EL (2014) Clinical Analysis of Sebum by Mass Spectrometry-A Brief Update. Clin Microbial 3: 138. doi:10.4172/2327-5073.1000138

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Separation	Advantage	Disadvantage
GC	Robust and versatile methodology for small molecules Wide dynamic range for valid quantification	Must derivatize lipid compounds: can be tedious/expensive Not suitable for large compounds
HPLC/UPLC	Wide dynamic range Allows separation of large molecules	Can require large volumes of solvent Can require long run times
TLC	Cheap and little technical experience required Can give quantitative values on broad lipid class if scanning densitometry is used	Unable to differentiate individual lipid molecules unless combined with other techniques
Solid phase extraction	More suited for large scale preparative separation Purifies lipids for downstream analysis	Unable to analyze lipid class or molecules unless combined with other techniques
Ionization	Advantage	Disadvantage
ESI	Quantitative Produces high proportions of singly charged ions with little fragmentation	Low salt tolerance
MALDI	More tolerant of salts and detergents Can be used in parallel with TLC or hard surface imaging	Matrix interferences Qualitative data collected Can produce multiple charged adducts
APCI	Can be used in tandem with ESI to increase amount of information collected	Higher proportions of fragments than ESI
EI	Rapidly identifies compounds compared to NIST library	Limited structural information available Harsh ionization
CI	Can be used to determine double bond position by MS/MS	Does not provide structure identification by single MS

Abbreviations: GC-gas chromatography; HPLC-high performance liquid chromatography; UPLC-ultra performance liquid chromatography; ESI-electrospray ionization; APCI-atmospheric pressure chemical ionization; EI-electron ionization; CI-chemical ionization.

Table 1: Common separation and ionization techniques associated with lipid mass spectrometry.

however, run times can be as long as one hour and the instability of the TMS esters increases time spent preparing samples if the GC/MS is equipped with an auto injector [12].

In conclusion, sebaceous lipids are a complex mixture of lipids and the composition can change from infectious microbes. Rapid analysis of sebum chemical composition can aid in clinical analysis of skin infections and drug development. Here we briefly outline some of the more classical methods of lipid analysis and collection of sebaceous lipids. New techniques in MS are rapidly expanding and the use of ESI tandem MS and MALDI-TOF MS have increased the versatility of lipid analysis.

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