Année 2023/2024 N°

Thèse

Pour le

DOCTORAT EN MEDECINE

Diplôme d'État

par

Nattie LECOLIER

Née le 6 octobre 1995 à Aix-en-Provence (13)

VALIDATION D'UNE PROCEDURE STANDARDISEE POUR L'ANALYSE METABOLOMIQUE ET LIPIDOMIQUE D'EMPREINTES CONJONCTIVALES HUMAINES

Présentée et soutenue publiquement le **31 mai 2024** devant un jury composé de :

Président du Jury : Professeur Pierre-Jean PISELLA, Ophtalmologie, Faculté de Médecine -Tours

Membres du Jury :

Professeur Hélène BLASCO, Biochimie et biologie moléculaire, Faculté de Médecine - Tours

Docteur Guillaume VANDERMEER, Ophtalmologie, PH, CHRU - Tours

Directeur de thèse : Docteur Raoul Kanav KHANNA, Ophtalmologie, PHU, CHRU - Tours

UNIVERSITE DE TOURS
FACULTE DE MEDECINE DE TOURS

DOYEN
Pr Patrice DIOT

VICE-DOYEN
Pr Henri MARRET

ASSESSEURS
Pr Denis ANGOULVANT, *Pédagogie*
Pr Mathias BUCHLER, *Relations internationales*
Pr Theodora BEJAN-ANGOULVANT, *Moyens – relations avec l'Université*
Pr Clarisse DIBAO-DINA, *Médecine générale*
Pr François MAILLOT, *Formation Médicale Continue*
Pr Patrick VOURC'H, *Recherche*

RESPONSABLE ADMINISTRATIVE
Mme Carole ACCOLAS

****

DOYENS HONORAIRES
Pr Emile ARON (†) – *1962-1966*
Directeur de l'Ecole de Médecine - 1947-1962
Pr Georges DESBUQUOIS (†) - *1966-1972*
Pr André GOUAZE (†) - *1972-1994*
Pr Jean-Claude ROLLAND – *1994-2004*
Pr Dominique PERROTIN – *2004-2014*

PROFESSEURS EMERITES
Pr Daniel ALISON
Pr Gilles BODY
Pr Philippe COLOMBAT
Pr Etienne DANQUECHIN-DORVAL
Pr Luc FAVARD
Pr Bernard FOUQUET
Pr Yves GRUEL
Pr Gérard LORETTE
Pr Loïc VAILLANT

PROFESSEURS HONORAIRES
P. ANTHONIOZ – P. ARBEILLE – A. AUDURIER – A. AUTRET – C. BARTHELEMY – J.L. BAULIEU – C. BERGER – JC. BESNARD – P. BEUTTER – C. BONNARD – P. BONNET – P. BOUGNOUX – P. BURDIN – L. CASTELLANI – J. CHANDENIER – A. CHANTEPIE – B. CHARBONNIER – P. CHOUTET – T. CONSTANS – C. COUET – L. DE LA LANDE DE CALAN – P. DUMONT – J.P. FAUCHIER – F. FETISSOF – J. FUSCIARDI – P. GAILLARD – G. GINIES – D. GOGA – A. GOUDEAU – J.L. GUILMOT – O. HAILLOT – N. HUTEN – M. JAN – J.P. LAMAGNERE – F. LAMISSE – Y. LANSON – O. LE FLOCH – Y. LEBRANCHU – E. LECA – P. LECOMTE – AM. LEHR-DRYLEWICZ – E. LEMARIE – G. LEROY – M. MARCHAND – C. MAURAGE – C. MERCIER – J. MOLINE – C. MORAINE – J.P. MUH – J. MURAT – H. NIVET – D. PERROTIN – L. POURCELOT – R. QUENTIN – P. RAYNAUD – D. RICHARD-LENOBLE – A. ROBIER – J.C. ROLLAND – P. ROSSET – D. ROYERE - A. SAINDELLE – E. SALIBA – J.J. SANTINI – D. SAUVAGE – D. SIRINELLI – J. WEILL

PROFESSEURS DES UNIVERSITES - PRATICIENS HOSPITALIERS

ANDRES Christian.....Biochimie et biologie moléculaire
ANGOULVANT Denis .....Cardiologie
APETOH Lionel .....Immunologie
AUPART Michel.....Chirurgie thoracique et cardiovasculaire
BABUTY Dominique .....Cardiologie
BACLE Guillaume.....Chirurgie orthopédique et traumatologique
BAKHOS David .....Oto-rhino-laryngologie
BALLON Nicolas.....Psychiatrie ; addictologie
BARBIER François.....Médecine intensive et réanimation
BARILLOT Isabelle .....Cancérologie ; radiothérapie
BARON Christophe .....Immunologie
BEJAN-ANGOULVANT Théodora .....Pharmacologie clinique
BERHOUET Julien .....Chirurgie orthopédique et traumatologique
BERNARD Anne .....Cardiologie
BERNARD Louis .....Maladies infectieuses et maladies tropicales
BLANCHARD-LAUMONNIER Emmanuelle ....Biologie cellulaire
BLASCO Hélène.....Biochimie et biologie moléculaire
BONNET-BRILHAULT Frédérique .....Physiologie
BOURGUIGNON Thierry .....Chirurgie thoracique et cardiovasculaire
BRILHAULT Jean.....Chirurgie orthopédique et traumatologique
BRUNEREAU Laurent .....Radiologie et imagerie médicale
BRUYERE Franck.....Urologie
BUCHLER Matthias.....Néphrologie
CAILLE Agnès .....Biostat., informatique médical et technologies de communication
CALAIS Gilles.....Cancérologie, radiothérapie
CAMUS Vincent.....Psychiatrie d'adultes
CORCIA Philippe.....Neurologie
COTTIER Jean-Philippe .....Radiologie et imagerie médicale
DEQUIN Pierre-François.....Thérapeutique
DESMIDT Thomas .....Psychiatrie
DESOUBEAUX Guillaume.....Parasitologie et mycologie
DESTRIEUX Christophe .....Anatomie
DI GUISTO Caroline .....Gynécologie obstétrique
DIOT Patrice.....Pneumologie
DU BOUEXIC de PINIEUX Gonzague .....Anatomie & cytologie pathologiques
DUCLUZEAU Pierre-Henri .....Endocrinologie, diabétologie, et nutrition
EHRMANN Stephan .....Médecine intensive – réanimation
EL HAGE Wissam.....Psychiatrie adultes
ELKRIEF Laure.....Hépatologie – gastroentérologie
ESPITALIER Fabien.....Anesthésiologie et réanimation, médecine d'urgence
FAUCHIER Laurent .....Cardiologie
FOUGERE Bertrand .....Gériatrie
FRANCOIS Patrick.....Neurochirurgie
FROMONT-HANKARD Gaëlle .....Anatomie & cytologie pathologiques
GATAULT Philippe.....Néphrologie
GAUDY-GRAFFIN Catherine.....Bactériologie-virologie, hygiène hospitalière
GOUPILLE Philippe .....Rhumatologie
GUERIF Fabrice.....Biologie et médecine du développement et de la reproduction
GUILLON Antoine.....Médecine intensive – réanimation
GUILLON-GRAMMATICO Leslie.....Epidémiologie, économie de la santé et prévention
GUYETANT Serge .....Anatomie et cytologie pathologiques
GYAN Emmanuel.....Hématologie, transfusion
HALIMI Jean-Michel.....Thérapeutique
HANKARD Régis.....Pédiatrie
HERAULT Olivier .....Hématologie, transfusion
HERBRETEAU Denis .....Radiologie et imagerie médicale
HOURIOUX Christophe.....Biologie cellulaire
IVANES Fabrice .....Physiologie
LABARTHE François .....Pédiatrie
LAFFON Marc .....Anesthésiologie et réanimation chirurgicale, médecine d'urgence
LARDY Hubert.....Chirurgie infantile
LARIBI Saïd.....Médecine d'urgence
LARTIGUE Marie-Frédérique .....Bactériologie-virologie
LAURE Boris.....Chirurgie maxillo-faciale et stomatologie
LECOMTE Thierry.....Gastroentérologie, hépatologie

LEGRAS Antoine.....Chirurgie thoracique
LESCANNE Emmanuel.....Oto-rhino-laryngologie
LEVESQUE Éric.....Anesthésiologie et réanimation chirurgicale, médecine d'urgence
LINASSIER Claude .....Cancérologie, radiothérapie
MACHET Laurent .....Dermato-vénéréologie
MAILLOT François .....Médecine interne
MARCHAND-ADAM Sylvain .....Pneumologie
MARRET Henri .....Gynécologie-obstétrique
MARUANI Annabel .....Dermatologie-vénéréologie
MEREGHETTI Laurent.....Bactériologie-virologie ; hygiène hospitalière
MITANCHEZ Delphine .....Pédiatrie
MOREL Baptiste.....Radiologie pédiatrique
MORINIERE Sylvain.....Oto-rhino-laryngologie
MOUSSATA Driffa .....Gastro-entérologie
MULLEMAN Denis.....Rhumatologie
ODENT Thierry.....Chirurgie infantile
OUAISSI Mehdi .....Chirurgie digestive
OULDAMER Lobna.....Gynécologie-obstétrique
PAINTAUD Gilles .....Pharmacologie fondamentale, pharmacologie clinique
PATAT Frédéric .....Biophysique et médecine nucléaire
PERROTIN Franck.....Gynécologie-obstétrique
PISELLA Pierre-Jean.....Ophtalmologie
PLANTIER Laurent.....Physiologie
REMERAND Francis .....Anesthésiologie et réanimation, médecine d'urgence
ROINGEARD Philippe.....Biologie cellulaire
RUSCH Emmanuel.....Epidémiologie, économie de la santé et prévention
SAINT-MARTIN Pauline.....Médecine légale et droit de la santé
SALAME Ephrem.....Chirurgie digestive
SAMIMI Mahtab .....Dermatologie-vénéréologie
SANTIAGO-RIBEIRO Maria .....Biophysique et médecine nucléaire
SAUTENET-BIGOT Bénédicte .....Thérapeutique
THOMAS-CASTELNAU Pierre .....Pédiatrie
TOUTAIN Annick.....Génétique
VELUT Stéphane.....Anatomie
VOURC'H Patrick.....Biochimie et biologie moléculaire
WATIER Hervé .....Immunologie
ZEMMOURA Ilyess .....Neurochirurgie

PROFESSEUR DES UNIVERSITES DE MEDECINE GENERALE

DIBAO-DINA Clarisse
LEBEAU Jean-Pierre

PROFESSEURS ASSOCIES

MALLET Donatien.....Soins palliatifs

PROFESSEUR CERTIFIE DU 2ND DEGRE

MC CARTHY Catherine.....Anglais

MAITRES DE CONFERENCES DES UNIVERSITES - PRATICIENS HOSPITALIERS

AUDEMARD-VERGER Alexandra.....Médecine interne
BISSON Arnaud.....Cardiologie (CHRO)
BRUNAULT Paul.....Psychiatrie d'adultes, addictologie
CARVAJAL-ALLEGRIA Guillermo.....Rhumatologie (au 01/10/2021)
CLEMENTY Nicolas.....Cardiologie
DOMELIER Anne-Sophie.....Bactériologie-virologie, hygiène hospitalière
DUFOUR Diane.....Biophysique et médecine nucléaire
FOUQUET-BERGEMER Anne-Marie.....Anatomie et cytologie pathologiques
GARGOT Thomas.....Pédopsychiatrie
GOUILLEUX Valérie.....Immunologie
HOARAU Cyrille.....Immunologie
KERVARREC Thibault.....Anatomie et cytologie pathologiques
LE GUELLEC Chantal.....Pharmacologie fondamentale, pharmacologie clinique
LEDUCQ Sophie.....Dermatologie
LEFORT Bruno.....Pédiatrie
LEJEUNE Julien.....Hématologie, transfusion
LEMAIGNEN Adrien.....Maladies infectieuses
MACHET Marie-Christine.....Anatomie et cytologie pathologiques
MOUMNEH Thomas.....Médecine d'urgence
PARE Arnaud.....Chirurgie maxillo-faciale et stomatologie
PIVER Éric.....Biochimie et biologie moléculaire
ROUMY Jérôme.....Biophysique et médecine nucléaire
STANDLEY-MIQUELESTORENA Elodie.....Anatomie et cytologie pathologiques
STEFIC Karl.....Bactériologie
TERNANT David.....Pharmacologie fondamentale, pharmacologie clinique
VAYNE Caroline.....Hématologie, transfusion
VUILLAUME-WINTER Marie-Laure.....Génétique

MAITRES DE CONFERENCES DES UNIVERSITES

AGUILLON-HERNANDEZ Nadia.....Neurosciences
NICOGLOU Antonine.....Philosophie – histoire des sciences et des techniques
PATIENT Romuald.....Biologie cellulaire
RENOUX-JACQUET Cécile.....Médecine Générale

MAITRES DE CONFERENCES ASSOCIES

AUMARECHAL Alain.....Médecine Générale
BARBEAU Ludivine.....Médecine Générale
CHAMANT Christelle.....Médecine Générale
ETTORI Isabelle.....Médecine Générale
MOLINA Valérie.....Médecine Générale
PAUTRAT Maxime.....Médecine Générale
PHILIPPE Laurence.....Médecine Générale
RUIZ Christophe.....Médecine Générale
SAMKO Boris.....Médecine Générale

CHERCHEURS INSERM - CNRS - INRAE

BECKER Jérôme.....Chargé de Recherche Inserm – UMR Inserm 1253
BOUAKAZ Ayache .....Directeur de Recherche Inserm – UMR Inserm 1253
BOUTIN Hervé.....Directeur de Recherche Inserm – UMR Inserm 1253
BRIARD Benoit.....Chargé de Recherche Inserm – UMR Inserm 1100
CHALON Sylvie .....Directrice de Recherche Inserm – UMR Inserm 1253
DE ROCQUIGNY Hugues .....Chargé de Recherche Inserm – UMR Inserm 1259
ESCOFFRE Jean-Michel.....Chargé de Recherche Inserm – UMR Inserm 1253
GILOT Philippe.....Chargé de Recherche Inrae – UMR Inrae 1282
GOMOT Marie.....Chargée de Recherche Inserm – UMR Inserm 1253
GOUILLEUX Fabrice .....Directeur de Recherche CNRS – EA 7501 - ERL CNRS 7001
GUEGUINOU Maxime.....Chargé de Recherche Inserm – UMR Inserm 1069
HEUZE-VOURCH Nathalie.....Directrice de Recherche Inserm – UMR Inserm 1100
KORKMAZ Brice.....Chargé de Recherche Inserm – UMR Inserm 1100
LATINUS Marianne.....Chargée de Recherche Inserm – UMR Inserm 1253
LAUMONNIER Frédéric .....Directeur de Recherche Inserm - UMR Inserm 1253
LE MERRER Julie .....Directrice de Recherche CNRS – UMR Inserm 1253
MAMMANO Fabrizio.....Directeur de Recherche Inserm – UMR Inserm 1259
MEUNIER Jean-Christophe .....Chargé de Recherche Inserm – UMR Inserm 1259
PAGET Christophe .....Directeur de Recherche Inserm – UMR Inserm 1100
RAOUL William .....Chargé de Recherche Inserm – UMR Inserm 1069
SECHER Thomas.....Chargé de Recherche Inserm – UMR Inserm 1100
SI TAHAR Mustapha .....Directeur de Recherche Inserm – UMR Inserm 1100
SUREAU Camille .....Directrice de Recherche émérite CNRS – UMR Inserm 1259
TANTI Arnaud .....Chargé de Recherche Inserm - UMR Inserm 1253
WARDAK Claire.....Chargée de Recherche Inserm – UMR Inserm 1253

CHARGES D'ENSEIGNEMENT

Pour l'éthique médicale
BIRMELE Béatrice.....Praticien Hospitalier

Pour la médecine manuelle et l'ostéopathie médicale
LAMANDE Marc .....Praticien Hospitalier

Pour l'orthophonie
BATAILLE Magalie.....Orthophoniste
CLOUTOUR Nathalie.....Orthophoniste
CORBINEAU Mathilde.....Orthophoniste
EL AKIKI Carole .....Orthophoniste
HARIVEL OUALLI Ingrid .....Orthophoniste
IMBERT Mélanie .....Orthophoniste
SIZARET Eva .....Orthophoniste

Pour l'orthoptie
BOULNOIS Sandrine.....Orthoptiste

SERMENT D'HIPPOCRATE

En présence des Maîtres de cette Faculté,
de mes chers condisciples
et selon la tradition d'Hippocrate,
je promets et je jure d'être fidèle aux lois de l'honneur et
de la probité dans l'exercice de la Médecine.

Je donnerai mes soins gratuits à l'indigent,
et n'exigerai jamais un salaire au-dessus de mon travail.

Admis dans l'intérieur des maisons, mes yeux
ne verront pas ce qui s'y passe, ma langue taira
les secrets qui me seront confiés et mon état ne servira pas à
corrompre les mœurs ni à favoriser le crime.

Respectueux et reconnaissant envers mes Maîtres, je
rendrai à leurs enfants
l'instruction que j'ai reçue de leurs pères.

Que les hommes m'accordent leur estime
si je suis fidèle à mes
promesses.
Que je sois couvert
d'opprobre et méprisé de mes
confrères
si j'y manque.

RESUME

Introduction : Les pathologies de la surface oculaire se manifestent par des symptômes et signes cliniques non spécifiques. L'empreinte conjonctivale (EC) est une méthode de prélèvement cellulaire rapide et non-invasive, ayant démontré son utilité dans le diagnostic et la compréhension des mécanismes physiopathologiques des maladies de la surface oculaire. L'approche métabo-lipidomique appliquée aux EC pourrait représenter une stratégie pertinente pour l'identification de biomarqueurs, mais nécessite une standardisation des procédures pré-analytiques et analytiques. L'objectif de cette étude était de valider les étapes pré-analytiques et analytiques de l'analyse métabo-lipidomique d'EC par chromatographie liquide ultra-haute-performance couplée à la spectrométrie de masse haute-résolution.

Matériel et méthodes : Quatre EC ont été réalisées successivement sur les yeux droits et gauches de 20 sujets sains, soit 160 empreintes. Trois protocoles d'extraction des métabolites (méthanol (MeOH), MeOH/eau et acétonitrile) et 2 protocoles d'extraction des lipides (méthyl tert-butyl éther et isopropanol (IPA)) ont été testés. Les métabolites et voies métaboliques identifiés dans les cellules conjonctivales ont été comparés avec ceux retrouvés dans les larmes humaines.

Résultats : Nous avons identifié 211 métabolites impliqués dans 9 voies métaboliques. Bien qu'il existe une variabilité importante des 4 empreintes successivement réalisées sur le même œil, les deux premières étaient comparables. Le métabolome conjonctival des deux yeux était comparable. La comparaison avec les 137 métabolites identifiés dans les larmes a mis en évidence 79 métabolites communs aux 2 matrices, 132 spécifiques aux EC et 58 spécifiques aux larmes. Avec l'identification de 262 lipides répartis en 24 classes lipidiques, la méthode d'extraction par IPA était la plus performante.

Conclusion : Ces résultats confirment la faisabilité de l'analyse métabo-lipidomique à partir d'EC et montrent une comparabilité interoculaire du métabolome conjonctival de sujets sains. La réalisation d'empreintes successives doit être prise en compte car elle est source de variabilité pré-analytique. Le métabolome des cellules conjonctivales est distinct et complémentaire de celui des larmes pour l'étude de la surface oculaire. Nous proposons donc une procédure standardisée pour l'approche métabo-lipidomique appliquée aux EC permettant la recherche de biomarqueurs en pratique clinique ophtalmologique.

Mots clés : métabolomique ; lipidomique ; empreinte conjonctivale ; spectrométrie de masse ; chromatographie liquide

ABSTRACT

TITLE: **VALIDATION OF A STANDARDIZED PROCEDURE FOR METABOLOMIC AND LIPIDOMIC ANALYSIS OF HUMAN CONJUNCTIVAL IMPRESSION CYTOLOGY SPECIMENS**

Introduction: Pathologies of the ocular surface are characterized by non-specific symptoms and clinical signs. Conjunctival impression cytology (IC) is a rapid and non-invasive cell sampling method that is useful for the diagnosis of ocular surface diseases and the understanding of the underlying pathophysiological mechanisms. The metabo-lipidomic approach applied to conjunctival IC could be a relevant strategy for biomarker identification but requires standardization of pre-analytical and analytical procedures. The objective of this study was to validate a workflow for metabo-lipidomic analysis of conjunctival IC specimens using ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry.

Methods: Four IC specimens were performed on both eyes of 20 healthy subjects, resulting in a total of 160 specimens. The study tested 3 different metabolite extraction protocols (methanol (MeOH), MeOH/water, and acetonitrile), as well as two lipid extraction protocols (methyl tert-butyl ether and isopropanol (IPA)). The metabolites and metabolic pathways identified in conjunctival cells were compared with those found in human tears.

Results: We identified 211 metabolites involved in 9 pathways. Although there was considerable variability in the 4 consecutive IC specimens performed on the same eye, the first two were comparable. The conjunctival metabolome of both eyes was comparable. Comparison of the 137 metabolites identified in tears and IC specimens revealed 79 common metabolites, while 132 were specific to IC specimens and 58 were specific to tears. The IPA extraction was the method of choice, enabling the identification of 262 lipids from 24 lipid classes.

Conclusion: These results confirm the feasibility of metabo-lipidomic analysis using IC specimens and demonstrate the comparability of the conjunctival metabolome between both eyes in healthy subjects. The use of successive IC is a source of variability. The metabolome of conjunctival cells differs from that of tears and can be used in conjunction with tears to study the ocular surface. To facilitate biomarker research in clinical practice, we propose a standardized procedure for the metabo-lipidomic analysis of conjunctival IC specimens.

Keywords: metabolomics; lipidomics; conjunctival impression cytology; mass spectrometry; liquid chromatography

ABBREVIATIONS

ACN: acetonitrile

AH: aqueous humor

DED: dry eye disease

ESI: electrospray ionization

FDR: false discovery rate

HILIC: hydrophilic interaction liquid chromatography

IC: impression cytology

IPA: isopropanol

LE: left eye

MeOH: methanol

MMP: mucous membrane pemphigoid

MTBE: methyl tert-butyl ether

MZ: mass-to-charge ratio

PES: polyether sulfone

RE: right eye

RT: retention time

QC: quality control

RT: retention times

UHPLC-HRMS: ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry

TABLE OF CONTENTS

INTRODUCTION

I. Conjunctival impression cytology for studying the ocular surface

The ocular surface refers to an anatomo-physiological entity located at the interface between the external environment and intraocular structures responsible for lubrification, protection against external physical aggressions and microorganisms as well as maintaining optimal visual performance. It includes the eyelids, lacrimal glands, Meibomian glands, tear film, conjunctiva, and cornea. Regarding the tear film, it is composed of three layers: a superficial lipid layer, primarily produced by the Meibomian glands; an intermediate aqueous layer mainly produced by the lacrimal glands (main and accessory); and an inner mucous layer adherent to the corneal epithelium primarily composed of mucins (**Figure 1**). The conjunctiva is a transparent mucous membrane covering the anterior surface of the eyeball and the posterior surface of the eyelids. Histologically, conjunctiva is a non-keratinized stratified epithelium, composed of 2 to 18 layers of cells depending on the location, being more prominent at the limbus and fornix (1).

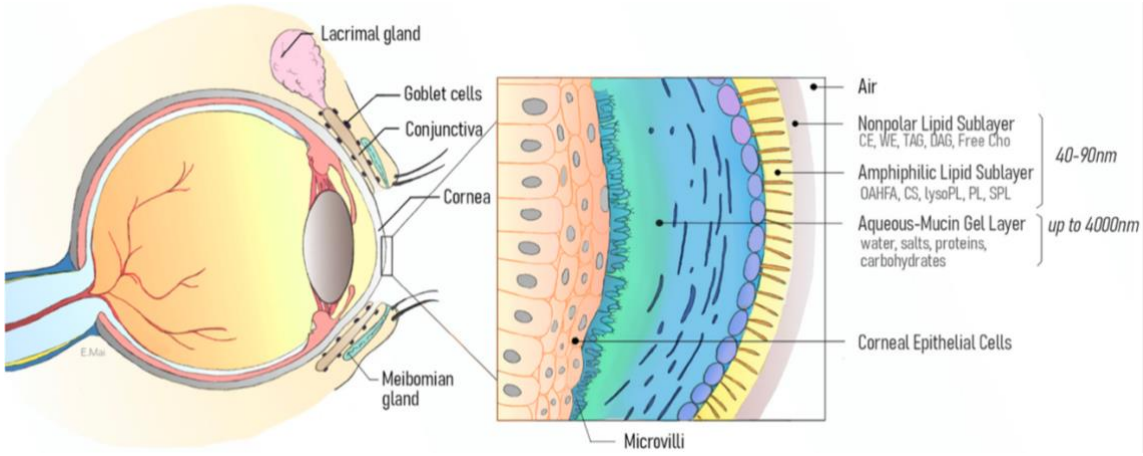

Figure 1. Schematic representation of human ocular surface components and tear film composition. CE: cholesterol ester; WE: wax ester; TAG: triacylglycerol; DAG: diacylglycerol; Free Cho: free cholesterol; OAHFA: (o-acyl)-ω-hydroxy fatty acids; CS: cholesteryl sulfate; PL: phospholipids; SPL: sphingophospholipids (2).

Conjunctival impression cytology (IC) is a technique of collecting cells from the superficial layers of the conjunctival epithelium by applying a cellulose acetate or polyether sulfone (PES) filter to the conjunctiva (**Figure 2**). First introduced in the late 1970s, its non-invasive, painless, inexpensive and rapid nature made it the ideal tool for clinical research into ocular surface pathology. It is also repeatable over time, making it useful for monitoring disease progression

or the effect of a treatment. Conjunctival IC has greatly contributed to the understanding of ocular surface pathologies, such as dry eye disease (3), allergic conjunctivitis (4), and ocular rosacea (5), particularly through the identification of overexpressed inflammatory and apoptosis-related biomarkers in conjunctival cells (6).

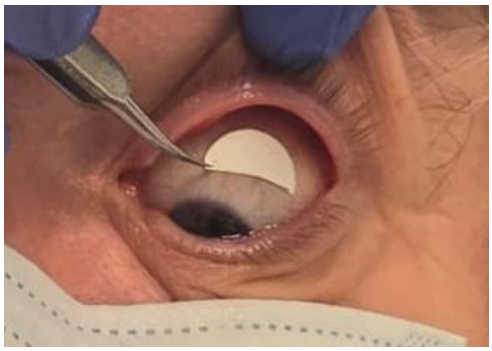

Figure 2. Photograph of a conjunctival impression cytology made on the superior bulbar conjunctiva.

II. Metabo-lipidomics: a high-throughput technology

Metabolomics is the process of identifying and quantifying low molecular weight molecules (< 1500 Daltons) present in biological fluids, cells, or tissues, in order to study the metabolome (7). Lipidomics corresponds to the identification and quantification of lipids within a matrix that collectively form the lipidome. Although some authors consider the lipidome as a component of the metabolome, its analysis is conducted distinctly from metabolites, due to distinct extraction methods and the low polarity of lipids compared to most water-soluble metabolites (8,9).

In medicine, these high-performance techniques can be used to identify pathology-specific biomarkers which are useful for improving diagnoses, understanding diseases pathophysiology, and identifying potential prognostic indicators or new therapeutic targets. Lastly, the biomarkers identified can be monitored in longitudinal studies and used to assess response to therapy (10). A good biomarker needs to be sensitive, specific, easily accessible via non or minimally invasive procedures that are feasible in clinical practice. Thus, superficial conjunctival cells are an ideal matrix for biomarker identification. The search for biomarkers is a promising field in ocular surface pathologies because specific symptoms and clinical signs are lacking, and diagnostic tests may not always correspond to the severity of the damage experienced by the patient (11).

III. CATARACTOMIQUE study: clinical application for biomarkers identification

CATARACTOMIQUE is a longitudinal clinical cohort study conducted at the regional university hospital center of Tours. The aim of the study is to identify predictive biomarkers of dry eye disease (DED) following cataract surgery on 100 subjects (ClinicalTrials.gov Identifier: NCT05802550). In this study, two IC specimens will be sampled from the eye to be operated on: one before the surgery and the other one a month after the surgery. One IC specimen will be used for metabolomic analysis and the other for lipidomic analysis using ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS).

Cataract is an opacification of the crystalline lens, most often associated with ageing and responsible for a reduction in visual acuity. It is the leading surgical procedure in France, with more than 800,000 operations performed each year (12). The results in terms of visual recovery are excellent, but some patients develop postoperative DED, a multi-factorial disease of the tears and ocular surface (13), resulting in fluctuating visual acuity, foreign body sensation, burning or even pain accompanied by photophobia and lacrimation. This has a significant impact on patients' vision related quality of life (14). The prevalence of DED at 4 weeks post-op varies from 9% to 31% depending on the study and has been reported to be 27% at 3 months post-op using the Ocular Surface Disease Index (OSDI) questionnaire (15). Although DED constitutes a worldwide public health challenge, its diagnosis is difficult because symptoms and signs are unspecific and in clinical practice there is a significant discrepancy between symptoms and signs of DED. Furthermore, despite the large number of diagnostic tests available to assess DED, most have low reliability and reproducibility (16,17), and there is no gold standard test to diagnose pre-operative ocular surface disorders (18). Thus, a patient who is asymptomatic preoperatively may have an early stage of the disease that could lead to true symptomatic DED after surgery. This highlights the relevance of a strategy to identify objective and reproducible biomarkers that predict the risk of DED prior to surgery, so that patients identified as “high risk” can be offered personalized management to increase their chances of successful surgery. Recently, an expanding body of research on tears indicates that altered metabolism plays an important role in the pathogenesis of DED (2), thus the identification of biomarkers could improve our understanding of the pathophysiology of ocular surface disorders following cataract surgery at the molecular level.

The aim of the current study was to develop and validate pre-analytical and analytical procedures for metabolomic and lipidomic analysis of IC-collected conjunctival cells using UHPLC-HRMS, for use in clinical research applications. We first explored the feasibility of extracting cellular metabolites and lipids from the solid and adherent PES membrane. Then, the reproducibility of the metabolome of successive IC specimens made on the same eye was assessed. The inter-eye metabolomic and lipidomic variability was also investigated. Metabolites and metabolic pathways identified in IC specimens were compared to those found in tears in a previous study conducted by our team (19). Finally, we compared 3 metabolite extraction solvents: acetonitrile (ACN), methanol (MeOH) and MeOH/water; and 2 lipid extraction solvents: isopropanol (IPA) and methyl tert-butyl ether (MTBE) (**Figure 3**).

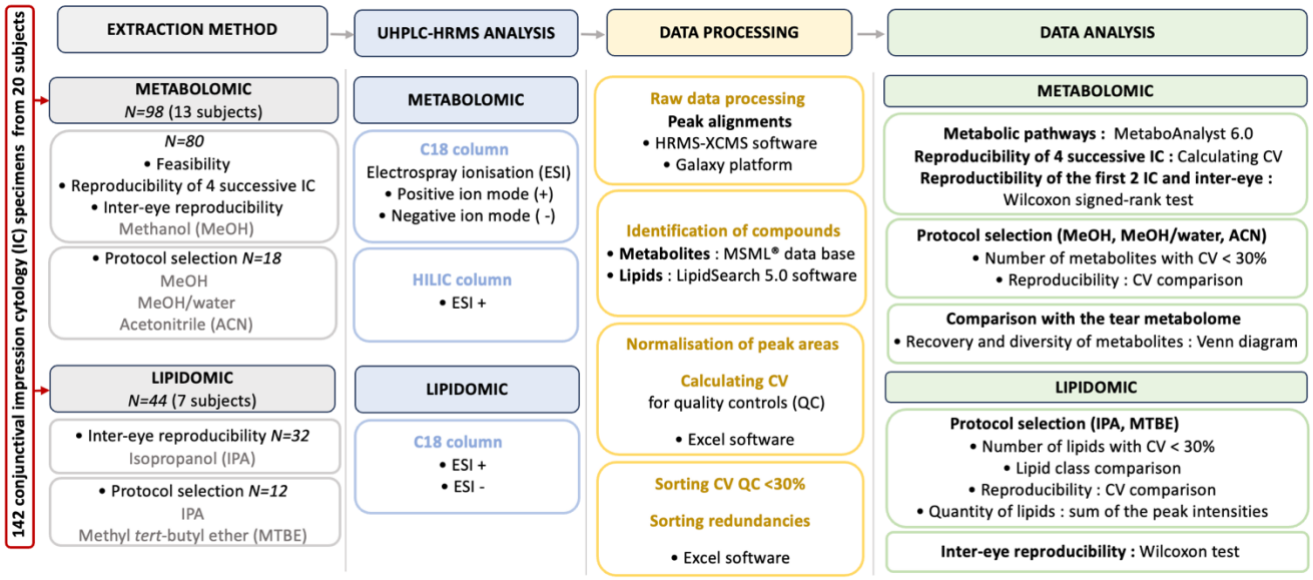

Figure 3. Schematic representation of the global workflow, including sample preparation, data analysis and the main evaluation parameters. CV: coefficients of variation; MSML: Mass Spectroscopy Metabolite Library.

MATERIAL AND METHODS

I. Origin, sampling method and storage of samples

Healthy volunteers were recruited from the ophthalmology department of the University Hospital of Tours, and all of them gave their informed consent. The protocol was approved by an independent Ethics Committee (ClinicalTrials.gov Identifier: NCT05802550). This study followed the tenets of the Declaration of Helsinki. The exclusion criteria were as follows: any history of systemic or ophthalmological disease, past or current ophthalmological symptoms, wearing contact lenses within the previous 48 hours, or instillation of eye drops within the last 24 hours. All included subjects were ≥18 years old.

After instillation of an ocular surface anesthetic eye drop (oxybuprocaine chlorhydrate 1,6 mg/0,4 mL, Théa©) and gentle traction of the upper eyelid, participants were instructed to look downwards. A PES filter paper disc with a diameter of 13mm and a pore size of 0.20µm cut in half (Supor®, Pall Life Sciences) was applied to the upper bulbar conjunctiva for 2 seconds on each side without exerting any pressure and then removed using disposable pre-sterilized forceps. In each eye, 4 filters were applied successively, labelled ABCD on the right eye (RE) and A'B'C'D' on the left eye (LE) (**Figure 4)**. The first two (AB and A'B') were made supra-temporally and the next two (CD and C'D') supra-nasally of the bulbar conjunctiva, shifting the area of application between each impression. They were taken by a single experienced ophthalmologist to minimize intra-operator variability and avoid inter-operator variability. Samples were placed in 0.5 ml Eppendorf tubes and immediately stored at -80°C until analysis.

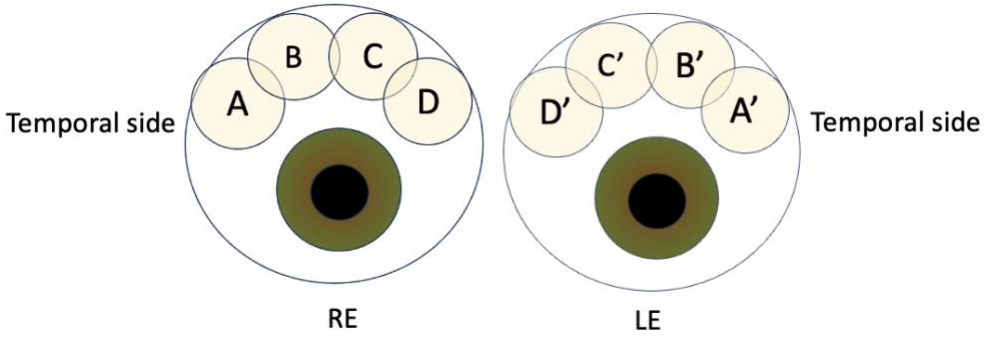

Figure 4. Conjunctival impression cytology: schematic representation of placement of filter papers to collect cells from bulbar conjunctiva of each eye. The application area has been shifted from the supratemporal zone (AB and A’B’) to the supranasal zone (CD and C’D’). LE: left eye; RE: right eye.

II. Compound extraction methods

1. Metabolite extraction protocols

We selected methanol (MeOH) as the extraction solvent because it has proven its efficiency for metabolite separation before mass spectrometry analysis in a prior study conducted by our team (20). One millilitre (mL) of pure MeOH was added on the PES filters, the mixture was homogenized by vortex, stirred for 1 hour on a planar stirrer and centrifuged at 15,000 g at 4°C for 10 minutes (min). Then 900 microlitre (µL) of supernatant was collected and split into two samples for analysis on a C18 column and on a hydrophilic interaction liquid chromatography (HILIC) column. Each was evaporated for 30 min at 40°C under nitrogen flow (Evaporex® EVX-96, Apricot Design). One hundred µL of MeOH/water (1:9, v/v) or acetonitrile/water (8:2, v/v) was added respectively for C18 and HILIC column analysis, the mixture was homogenized by vortexing and transferred to chromatograph-compatible vials for analysis.

Then, two additional metabolite extraction methods were tested: MeOH/water and ACN and compared with pure MeOH. One mL of MeOH/water (1:1, v/v) or ACN was added on the PES filters, and the same protocol as previously described was followed.

Quality control (QC) samples were prepared from a pool of equal volumes (5 µL) of all samples extracted using the same protocol.

2. Lipid extraction protocols

Two methods of extracting lipids were evaluated: isopropanol (IPA) and methyl tert-butyl ether (MTBE).

IPA extraction involved adding 1 mL of pure IPA. The mixture was vortexed for 5 seconds (s), stirred for 1 hour on a planar stirrer, and centrifuged at 15,000 g, at 4°C, for 15 min. A single-phase solution was obtained, from which 900 µL was recovered and transferred to glass tubes for 30 min solvent evaporation at 40°C under nitrogen flow (Evaporex® EVX-96, Apricot Design).

MTBE extraction involved adding 500 µL of Milli-Q® water and 500 µL of MTBE. The mixture was vortexed for 1 min, stirred for 1 hour on a planar stirrer, and centrifuged at 15,000 g, at 4°C, for 15 min. A two-phase solution was obtained and 450 µL of the upper phase

was recovered and transferred to a 96-well plate for 30 min solvent evaporation under nitrogen flow at 40°C (Evaporex® EVX-96, Apricot Design).

For both protocols, the dry residues were re-suspended in 100 µL of a 60:35:5, v/v/v, mix of acetonitrile/IPA/water, then transferred to chromatograph-compatible vials for analysis.

Quality control samples were prepared from a pool of equal volumes (5 µL) of all samples extracted using the same protocol.

III. Reproducibility of metabolome in successive IC specimens from the same eye

Eighty IC specimens from 10 subjects (labelled S1 to S10) were extracted for metabolites using MeOH to assess the reproducibility of metabolome in successive IC specimens made sequentially on the same eye of the same subject (**Table 1**).

	RE	**LE**
S1	ABCD	A'B'C'D'
S2	ABCD	A'B'C'D'
S3	ABCD	A'B'C'D'
S4	ABCD	A'B'C'D'
S5	ABCD	A'B'C'D'
S6	ABCD	A'B'C'D'
S7	ABCD	A'B'C'D'
S8	ABCD	A'B'C'D'
S9	ABCD	A'B'C'D'
S10	ABCD	A'B'C'D'

Table 1. Subjects and samples dedicated to reproducibility of metabolome assessment in successive IC specimens from the same eye and inter-eye comparison. IC: impression cytology; LE: left eye; RE: right eye; S: subject.

The comparison focused on the relative quantity of the extracted metabolites in successive IC specimens, achieved by comparing the area under the curve of their chromatographic peaks (*i.e.* the intensities). For each detected metabolite, for each eye (RE then LE) and for each subject, the coefficient of variation (CV% = (the standard deviation/the mean) x 100)) for the peak's intensities of the 4 IC specimens (ABCD then A'B'C'D) was calculated using Excel software. The mean CV obtained from the 10 subjects was then calculated to obtain a single CV per metabolite. The acceptable threshold for variability was set at 30%, as chosen in a previous study for basal tears metabo-lipidomics (19).

The comparison of the first two IC specimens (AB and A'B') was performed using a paired non-parametric univariate statistic test (Wilcoxon's rank-sum test), which compared the intensities metabolite by metabolite, that is, the relative amount of extracted metabolites.

IV. Comparison of inter-eye metabo-lipidome

The same 80 methanol-extracted IC specimens from 10 subjects were used to assess inter-eye metabolome variability (**Table 1**).

Thirty-two IC specimens from 4 subjects (labelled S11 to S14) were extracted with IPA to assess inter-eye lipidome variability (**Table 2**).

	RE	**LE**
S11	ABCD	A'B'C'D'
S12	ABCD	A'B'C'D'
S13	ABCD	A'B'C'D'
S14	ABCD	A'B'C'D'

Table 2. Subjects and IC specimens dedicated to inter-eye lipidome variability assessment. IC: impression cytology; LE: left eye; RE: right eye; S: subject.

The metabolomic and lipidomic profile comparison between the RE (IC specimens ABCD) and the LE (IC specimens A'B'C'D') was performed using a paired non-parametric univariate statistic test (Wilcoxon's rank-sum test). A pairwise comparison of metabolite intensities was conducted on specimens of the same rank: A with A', B with B', C with C', and D with D'.

V. Selection of the optimal extraction solvent for metabolites and lipids

Eighteen IC specimens from 3 subjects (labelled S15 to S17) were used to determine the optimal solvent for metabolite extraction: ACN, MeOH, or MeOH/water. The metabolites of the IC specimens A from the RE of the 3 subjects were extracted using ACN and compared with those of the IC specimens A' from the LE of the same 3 subjects extracted using MeOH. The 3 specimens B extracted using MeOH were compared with the 3 specimens B' extracted using MeOH/water. Finally, the specimens C extracted using MeOH/water were compared with the specimens C' extracted using ACN (**Table 3**).

Tested solvents	IC samples used	Tested solvents	IC samples used	Tested solvents	IC samples used
ACN	S15_RE_A S16_ RE_A S17_RE_A	**MeOH**	S15_RE_B S16_ RE_B S17_RE_B	**MeOH/eau**	S15_RE_C S16_ RE_C S17_RE_C
MeOH	S15_LE_A' S16_LE_A' S17_LE_A'	**MeOH/Eau**	S15_LE_B' S16_LE_B' S17_LE_B'	**ACN**	S15_LE_C' S16_LE_C' S17_LE_C'

Table 3. Subjects and IC specimens for selecting of the optimal metabolite extraction solvent. IC: impression cytology; LE: left eye; MeOH: methanol; RE: right eye; S: subject.

To determine the most appropriate solvent for lipid extraction, either IPA or MTBE, 12 IC specimens from 3 subjects (labelled S18 to S20) were taken. The lipids extracted from the first and second IC specimens of the RE (AB) using IPA were compared with those extracted from the first and second IC specimens of the LE (A'B') using MTBE (**Table 4**).

Tested solvents	IC samples used
IPA	**S18**_RE_A B **S19**_RE_A B **S20**_RE_A B
MTBE	**S18**_LE_A'B' **S19**_LE_A'B' **S20**_LE_A'B'

Table 4. Subjects and IC specimens for selecting the optimal lipid extraction solvent. IC: impression cytology; IPA: isopropanol; LE: left eye; MTBE: methyl tert-butyl ether; RE: right eye; S: subject.

The optimal extraction solvents for both metabolites and lipids were selected based on the number of detected compounds with a CV below 30% and the reproducibility of the extracted metabolome and lipidome. The mean CV of the compounds was calculated for each solvent, and the compounds were then classified into four groups based on their CV: those with a CV between 20% and 30%, those with a CV between 10% and 20%, those with a CV between 5% and 10%, and those with a CV below 5%.

Finally, we compared the relative quantity of lipids extracted by each solvent by calculating the sum of the areas under the curve of the non-normalized chromatographic peaks using Excel software. To obtain a single value per solvent, the means of the sums of peak intensities extracted by each solvent were calculated, along with their standard deviation.

VI. Tear and conjunctival IC specimens: a metabolome comparison

Metabolites extracted from conjunctival IC specimens were compared to those found in tears in a previous study conducted by our team (21). Catanese *et al.* identified 137 metabolites in 10 µL samples of commercial human tears pooled from 3 anonymous donors purchased from MyBiosource® (San Diego, California, USA). Redundant and unique metabolites were identified in each of the two matrices using a Venn diagram. The metabolic pathways present in tears were analyzed and compared with those present in conjunctival IC specimens.

VII. Statistical analyses

A paired non-parametric univariate statistical test (Wilcoxon's rank-sum test) was used to compare the metabolome of both eyes and the first two IC specimens sampled from the same eye. The analysis was performed using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/). To detect significant differences in the areas under the curve of the chromatographic peaks, a significance level of 0.05 was selected, with a correction for multiple testing using the false discovery rate (FDR). A paired fold change analysis was conducted using a threshold of 1 and a comparison direction of A/B (then A'/B') to determine the relative abundance of each metabolite with significantly different intensities between the first two IC specimens. This was done to establish whether each metabolite was more abundant in the first or second IC specimen. Metabolic pathways in the IC specimens and tears were analyzed using MetaboAnalyst 6.0 pathway analysis. A metabolic pathway was considered significantly present if at least 20% of the metabolites in the pathway were present in the sample, with a p-value corrected by FDR of less than 0.05, and if its impact was at least 20%. Venn diagrams were used to identify similarities and differences in the metabolomic profiles of IC specimens and tears. Diagrams were created using the website http://jvenn.toulouse.inra.fr/app/example.html (22). To identify the optimal solvent for lipid extraction, we compared the lipid intensities extracted with IPA and MTBE through a paired non-parametric univariate statistical test (Wilcoxon's rank-sum test) with a p-value of 0.05, corrected using the FDR method in GraphPad Prism 9.0.0.

VIII. Mass spectrometry analyses

1. Metabolomics application

Metabolite profiling was performed in a targeted and semi-quantitative manner using a UHPLC Ultimate WPS-3000 system (Dionex, Germany) connected to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

Liquid chromatography was first performed by injecting 5 µL of the preparation into a phenomenex Kinetex HILIC column (150 mm × 2.10 mm, 100 Å) operating in positive electrospray ionization mode (ESI+). The gradient was maintained at a flow rate of 0.5 mL/min. The solvent system consisted of mobile phase A: [water + 10 mM ammonium formate, 0.5% formic acid] and mobile phase B: [ACN + 10 mM ammonium formate, 0.5% formic acid]. The multistep gradient was programmed as follows: 0-0,9 min, 95 % B; 0,9-4,8 min, 95-82 % B; 4,8-9 min, 82-10 % B; 9-11,4 min, 10-95 % B; 11,4-13,7 min, 95-95% B.

Then, 5 µL of the prepared solution were injected into a C18 UHPLC column operating in both ESI+ and ESI- modes (Phenomenex Kinetex 1.7 µm XB—C18 column; 100 mm × 2.10 mm) and maintained at 40 °C. The solvent system consisted of mobile phase A: [water + 0,1 % formic acid] and mobile phase B: [ACN + 0.1 % formic acid]. The gradient operated at a flow rate of 0.5 mL/min. The multistep gradient was set as follows: 0–2 min, 2 % B; 2–6 min, 2-30 % B; 6–9 min, 30–75% B; 9–9,5 min, 75–100% B; 9,5-11,8 min, 100-100% B; 11,8-12 min, 100-2% B; 12-13,7 min, 2-2% B.

2. Lipidomics application

Lipid profiling was performed in a targeted and semi-quantitative manner using a UHPLC Ultimate WPS-3000 system (Dionex, Germany) connected to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) operated in ESI+ and ESI- modes. Chromatography was performed utilizing a C18 UHPLC column (1.7 µm XB—C18, 150 mm × 2.10 mm, 100 Å) from Kinetex, Phenomenex, Torrance, CA, maintained at 55 °C. The detailed methodology is available in a prior publication (23). Two mobile phase gradients were used: A [isopropanol/ACN (9:1) + 0.1% (vol/vol) formic acid + 10 mM ammonium formate], and B [ACN/water (6:1) + 0.1% (vol/vol) formic acid + 10 mM ammonium formate]. The gradient was maintained at a flow rate of 0,26 mL/min. The multisteps gradient was programmed as follows: 0-1.5 min, 32-45 % A; 1.5-5min, 45-52 % A; 5-8min, 52-58% A; 8-

11min, 58-66% A; 11-14 min, 66-70 % A; 14-18min, 70-75 % A; 18-21 min, 75-97 % A; 21-24 min, 97 % A. The injection volume for each sample was 5 µL.

Instrumental stability was assessed by multiple injections (n=9 for metabolomic analysis and n=5 for lipidomic analysis) of a QC sample obtained from a pool of 5 µL of all samples extracted with the same solvent. For metabolomic analysis, a QC sample was injected once at the beginning of the analysis, between every 10 sample injections, and at the end of the run. For lipidomic analysis, two QC samples were injected at the beginning of the analysis, one at the midpoint, and two at the end of the run.

IX. Data processing following acquisition

The raw data was converted to mzXML format using MSConvert software and imported into HRMS-XCMS software (Sciex, Framingham, Massachusetts, USA) on the Galaxy platform (https://workflow4metabolomics.org) for extracting the mass-to-charge ratio/retention time pair (MZ/RT) with a detection intensity threshold of 10000 (arbitrary units) and for chromatogram alignment. Chromatograms from the same extraction solvent were analyzed together. For metabolites identification, we applied a targeted analysis based on a library of standard compounds (Mass Spectroscopy Metabolite Library (MSML®) of standards, IROA Technologies™, Bolton, Massachusetts, USA) covering 609 metabolites. The metabolites were identified using the following criteria: the metabolite's retention time had to be within ±20 s of the standard reference and its measured molecular mass had to be within a range of 10 ppm deviation from the known molecular mass of the reference compound. Additionally, there needed to be an accurate visual correspondence between the metabolite's isotopic ratios and those of the standard reference. Lipids were identified using LipidSearch™ 5.0 software (Thermo Fisher Scientific, Bremen, Germany) based on in silico ms/ms fragmentation.

In an Excel® table with metabolites rows and samples columns, peaks were normalized based on the total area. CV of normalized peak areas were computed for QC and expressed as percentage. Only metabolites with a CV lower than 30% were retained. For metabolomic analysis, redundancies among the different modalities (C18 mode +, C18 mode - and HILIC) were eliminated using retention times (RT): the metabolite with a RT greater than 60 s was retained. If all the metabolites had a RT greater than 60 s, the one with the lowest CV was selected. For lipidomic analysis, if there were redundancies between C18 mode + and –, we retained the modality with the lowest CV.

Table 5 summarizes the study's objectives and the distribution of conjunctival IC specimens used to achieve them.

Analysis	**Objectives**	**Number of sampled subjects *(numbering)***	**Number of IC specimens collected**	**Number of IC specimens used**
Metabolomic	Reproducibility of successive IC samples Inter-eye variability	10 *(S1-S10)*	80	80
	Selection of the optimum extraction solvent	3 *(S11-S13)*	24	18
Lipidomic	Inter-eye variability	4 *(S14-S17)*	32	32
	Selection of the optimum extraction solvent	3 *(S17-S20)*	24	12
Total		**20 *(S1-S20)***	**160**	**142**

Table 5. Presentation of the total number of conjunctival IC specimens sampled and their distribution to meet the different objectives of the study. IC: impression cytology; S: subject.

RESULTS

I. Population and sampling tolerance

Twenty subjects (median age: 23; minimum: 19; maximum: 31; male to female ratio: 0.25) were included in the study. During sampling, 5 subjects reported transient ocular pain. This was always reported at the last or penultimate sampling and was relieved by instilling an additional drop of oxybuprocaine hydrochloride. No participant reported ocular pain during the first two IC collections. No complications were reported following IC sampling, although some subjects experienced a prickling sensation and slight ocular redness, which was relieved by the instillation of artificial tears.

II. Metabolic contents

Using a C18 column in ESI- mode, 70 metabolites were identified; using a C18 column in ESI+ mode, 74 metabolites were identified; and using a HILIC column in ESI+ mode, 127 metabolites were identified. After excluding molecular redundancies and metabolites with a CV for QC over 30%, 211 metabolites were selected. The metabolomic profile of IC specimens, including the types of metabolites and their CV for CQ, is presented in **Supplemental table 1** (see page 47).

III. Poor reproducibility of successive IC specimens collected from the same eye

Assuming a variability threshold of 30% for the CV of the metabolite chromatographic peak intensities, only 85 and 59 metabolites out of the 211 identified were reproducible between the 4 sequential IC specimens for the right and left eye, respectively. When considering the first 3 IC specimens, the results were similar, with only 97 and 63 metabolites reproducible for the right and left eye, respectively (**Table 6**).

	ABCD	ABC	A'B'C'D'	A'B'C'
Number of metabolites with CV < 30% *(expressed in %)*	**85** / 211 *(40.3)*	**97** / 211 *(46)*	**59** / 211 *(28)*	**63** / 211 *(29.9)*
Mean CV of all metabolites (%)	37.9	34.1	40.9	40.3
Mean CV of metabolites with CV < 30% (%)	21.7	20.4	22.6	22.1

Table 6. Number of metabolites with a coefficient of variation (CV) for peak intensities below 30% when performing 4 or 3 consecutive conjunctival impression cytology on the right (ABCD) and left eye (A'B'C'D') and mean CV.

For more than 50% of the metabolites, a CV greater than 30% was obtained. Therefore, when performing 3 or 4 successive IC samples, the relative quantity of detected metabolites is not reproducible. The following section deals with the reproducibility of metabolite chromatographic peak intensities between the first two IC specimens (AB and A'B').

IV. A similar metabolic profile for the first two IC specimens

The paired non-parametric univariate statistical test (Wilcoxon's rank-sum test) conducted on the 211 identified metabolites did not reveal any statistically significant differences in metabolite peak intensities between the first two IC specimens for 194 metabolites in the right eye (*i.e.* 91.9% of the metabolites) and for 185 metabolites in the left eye (*i.e.* 87.7% of the metabolites). **Tables 7 and 8** list the significantly different metabolites.

Metabolites	p-value
Adenine	0.041211
Hippuric acid	0.041211
Hydroxyphenyl lactic acid	0.041211
5'-Methylthioadenosine	0.041211
Adenosine	0.041211
Pyridoxamine	0.041211
Urate	0.041211
Citicoline	0.041211
Citrulline	0.041211
SM (36_2)	0.041211
Allantoin	0.048483
Alpha-glucose	0.048483
Glycerate	0.048483
Indoxyl sulfate	0.048483
Succinic acid	0.048483
Diaminopimelic acid	0.048483
L-arginine	0.048483

Table 7. Metabolites with significantly different peak intensities between the first and second IC specimens on the right eye, with their p-values corrected by FDR. SM: sphingomyelin.

Metabolites	p-value
ADP-glucose	0.029436
Gluconic acid	0.029436
Glycerate	0.029436
Indoxyl sulfate	0.029436
L-cystine	0.029436
Succinic acid	0.029436
LysoPC(16_0)	0.029436
LysoPC(16_1)	0.029436
LysoPC(18_0)	0.029436
LysoPC(18_1)	0.029436
LysoPC(18_2)	0.029436
LysoPC(20_2)	0.029436
LysoPC(20_3)	0.029436
PC(O-12_0__2_0)	0.029436
Betaine	0.047551
LysoPC(14_0)	0.047551
Sorbate	0.047551
Hippuric acid	0.047551
Hydroxyphenyl lactic acid	0.047551
Pyroglutamic acid	0.047551
Ophtalmic acid	0.047551
Pyridoxamine	0.047551
LysoPE(18_0)	0.047551
LysoPI(18_0)	0.047551
LysoPI(18_0)	0.047551
SM(40_2)	0.047551

Table 8. Metabolites with significantly different peak intensities between the first and second IC specimens on the left eye, with their p-values corrected by FDR. ADP: adenosine triphosphate; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; SM: sphingomyelin.

Looking at the 17 metabolites which intensities differed significantly between A and B on the right eye (**table 7**), paired fold change analysis revealed an A/B ratio greater than 1 for only one metabolite; therefore, the remaining 16 metabolites were present in greater quantity in the B sample (**table 9**). For the 26 metabolites which intensities differed significantly between A’ and B’ on the left eye (**table 8**), paired fold change analysis revealed an A'/B' ratio greater than 1 for 4 metabolites; thus the remaining 22 metabolites were present in greater quantity in the B’ sample (**Table 9**).

With approximately 90% of the metabolites showing no significant difference in relative intensities, we can conclude that the first two IC specimens carried out on the same eye are comparable. For the remaining 10% of metabolites, the relative intensity is overall higher in the second sample (B and B’) than in the first (A and A’).

Metabolites	Fold change A/B
Indoxyl sulfate	**0,31**
Alpha-glucose	**0,34**
Adenosine	**0,36**
Allantoin	**0,36**
Diaminopimelic acid	**0,39**
Glycerate	**0,4**
Adenine	**0,42**
Hippuric acid	**0,46**
L-Arginine	**0,46**
Urate	**0,46**
Pyridoxamine	**0,5**
Succinic acid	**0,53**
Citrulline	**0,56**
5'-Methylthioadenosine	**0,6**
Hydroxyphenyllactic acid	**0,63**
SM(36_2)	**0,74**
Citicoline	**2,92**

Metabolites	Fold change A'/B'
L-Cystine	**0,28**
Indoxyl sulfate	**0,29**
LysoPC(20_2)	**0,32**
Hippuric acid	**0,36**
Glycerate	**0,37**
LysoPC(20_3)	**0,39**
LysoPC(18_1), LysoPE(18_0)	**0,4**
LysoPC(18_0)	**0,41**
LysoPC(16_0), PC(O-12_0_2_0)	**0,42**
LysoPC(16_1), LysoPI(18_0)	**0,43**
LysoPC(18_2)	**0,45**
Succinic acid	**0,48**
Sorbate	**0,5**
Hydroxyphenyllactic acid	**0,55**
Pyridoxamine	**0,55**
Betaine	**0,56**
LysoPC(14_0)	**0,57**
Pyroglutamic acid	**0,64**
SM(40_2)	**0,67**
Gluconic acid	**1,3**
Phosphorylcholine	**1,45**
Ophtalmic acid	**1,53**
ADP-glucose	**1,74**

Table 9. Fold change analysis of metabolites with significantly different peak intensities between the first two impression cytology specimens on the right eye (AB) and left eye (A'B'). Threshold = 1; Comparison direction = A/B (and A'/B').
ADP: adenosine triphosphate; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; SM: sphingomyelin.

V. No difference in metabolomic profile between eyes

The paired non-parametric univariate statistical test (Wilcoxon's rank-sum test) conducted on the 211 identified metabolites which were common between both eyes and comparing pairwise IC specimens of the same rank (A paired with A', B paired with B', C paired with C' and D paired with D') did not reveal any statistically significant differences in metabolite intensities between the right and left eyes.

The comparison of the lipidome between the two eyes is in progress. The same statistical test will be used.

VI. Metabolic pathway analysis

The MetaboAnalyst pathway analysis showed that these 211 metabolites were involved in 54 pathways, of which 9 were statistically significant based on the previously established criteria (*i.e.* at least 20% of the metabolites in the pathway were present in the sample, with a p-value corrected by FDR of less than 0.05, and an impact of at least 20%). The 9 statistically significant pathways identified in the IC specimens were : arginine biosynthesis pathway ($p=2,8\cdot10^{-7}$, impact=0,37), alanine, aspartate and glutamate metabolism pathway ($p=1,8\cdot10^{-5}$, impact=0,47), purine metabolism pathway ($p=7\cdot10^{-5}$, impact=0,33), phenylalanine, tyrosine and tryptophan biosynthesis pathway ($p=8\cdot10^{-4}$, impact=1), beta-alanine metabolism pathway (p=0,001, impact=0,4), arginine and proline metabolism pathway (p=0,001, impact=0,35), histidine metabolism pathway (p=0,001, impact=0,39), glycine, serine and threonine metabolism pathway (p=0,02, impact=0,54), and citrate cycle (p=0,05, impact=0,2).

VII. A complementary metabolism with that of tears

Seventy-nine common metabolites were detected both in tears and IC specimens. One hundred thirty-two were specific to IC specimens, and 58 to tears (**Figure 5**). Therefore, under physiological conditions, these two matrices appear to provide different and complementary information for studying the ocular surface, although a greater number of metabolites are detected in IC specimens.

Pathway analysis of the 137 metabolites extracted from tears revealed 49 pathways, of which only one was found to be significant: the biosynthesis of phenylalanine, tyrosine, and tryptophan ($p=1,5\cdot10^{-4}$, impact=1,0).

Overall, 79 out of the 137 metabolites identified in tears were also present in the IC specimens, and the unique metabolic pathway found in tears was also identified in IC specimens. Therefore, the IC samples showed significant expression of 8 additional pathways, thereby demonstrating their considerable potential for biomarker identification.

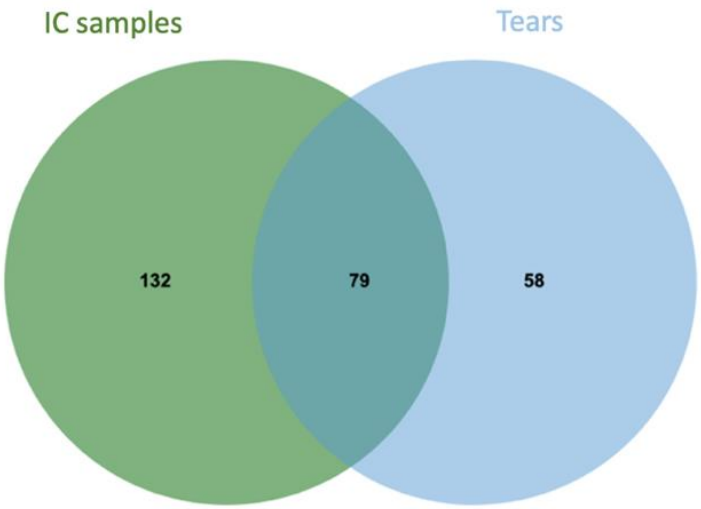

Figure 5. Venn diagram analysis. Comparison of number of metabolites with CV QC < 30% detected from IC samples and 10 µL of tears. 79 were common, 132 and 58 were specific, respectively. CV: coefficient of variation; QC: quality control; IC: impression cytology.

VIII. Lipidic contents

In the lipidomic analysis, using the C18 column in ESI- and ESI+ mode, 262 lipids with a CV QC below 30% from 24 classes were detected after extraction with IPA, and 263 lipids with a CV CQ below 30% from 22 classes were detected after extraction with MTBE.

In total, using these two extraction solvents, 25 lipid classes were identified in IC specimens. The percentage distribution of lipid classes extracted using IPA and MTBE is shown in **Figure 6**.

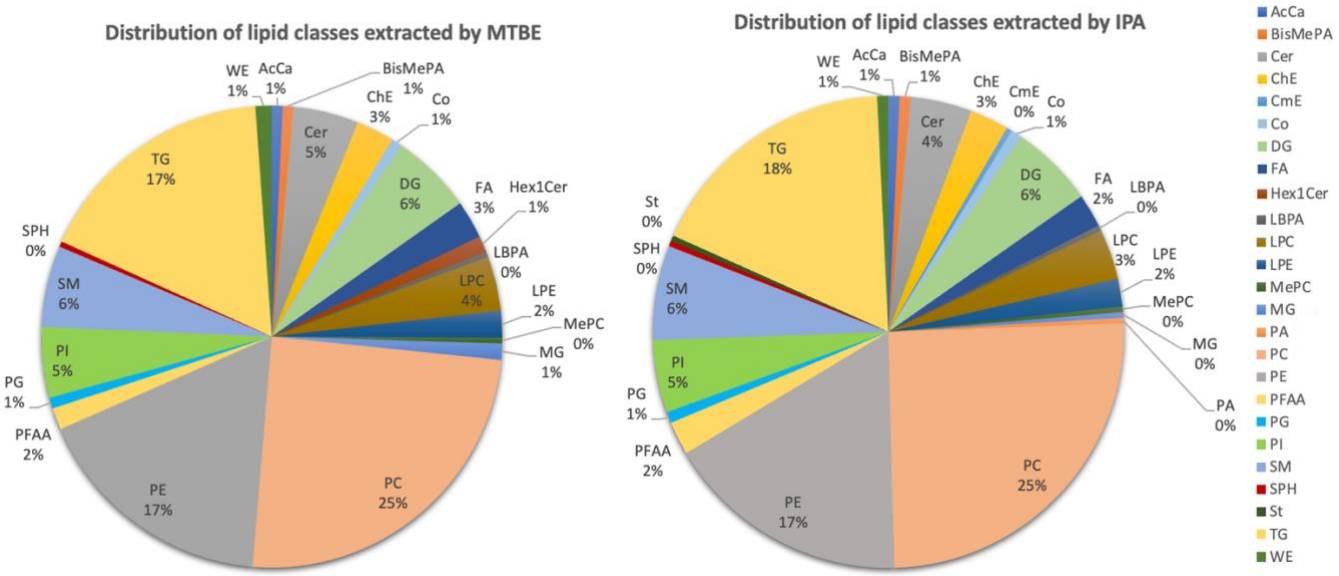

Figure 6. Distribution of lipid classes extracted by MTBE and IPA from IC specimens. AcCA: fatty acyl carnitine; BisMePA: bis-methyl phosphatidic acid; Cer: ceramide; ChE: cholesteryl ester; Cme: sterol esters; Co: Coenzyme Q; DG: diacylglycerol; FA: fatty acid, Hex1Cer: monoexosylceramide; LBPA: lysobisphosphatidic acid; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; MePC: methylphosphatidylcholine; MG: monoacylglycerol; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PFAA: primary amide; PG: phosphatidylglycerol; PI: phosphatidylinositol; SM: sphingomyelin; SPH: sphingosine; St: sterol; TG: triglyceride; WE: wax ester.

IX. Determination of lipid extraction solvent

The number of lipids extracted with a CV CQ below 30% was similar for both IPA and MTBE, with 262 and 263 lipids respectively. The distribution of this lipids into lipidic classes was also similar (**Figure 6**).

Out of the 25 identified lipid classes, 21 were found with both solvents. The number of metabolites within these common classes did not differ by more than 2 lipids between the two solvents (**Table 10**). Three lipid classes were exclusively found with IPA: sterol esters (CmE), phosphatidic acid (PA), and sterols (St). Only one lipid class was exclusively extracted by MTBE: monoexosylceramide (Hex1Cer) (**Table 10**).

	Lipid number	
	IPA	**MTBE**
PC	66	65
TG	46	45
PE	44	45
SM	17	15
DG	15	15
PI	13	13
Cer	11	12
LPC	9	10
ChE	7	7
FA	6	7
PFAA	6	4
LPE	5	6
Hex1Cer		3
AcCa	2	2
BisMePA	2	2
Co	2	2
PG	2	2
WE	2	3
CmE	1	
LBPA	1	1
MePC	1	1
MG	1	3
PA	1	
SPH	1	1
St	1	
Total	**262**	**263**

Table 10. Comparison of lipid classes and lipid molecule count detected based on extraction solvent: IPA of MTBE. AcCA: fatty acyl carnitine; BisMePA: bis-methyl phosphatidic acid; Cer: ceramide; ChE: cholesteryl ester; CmE: sterol ester; Co: Coenzyme Q; DG: diacylglycerol; FA: fatty acid, Hex1Cer: monoexosylceramide; LBPA: lysobisphosphatidic acid; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; MePC: methylphosphatidylcholine; MG: monoacylglycerol; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PFAA: primary amide; PG: phosphatidylglycerol; PI: phosphatidylinositol; SM: sphingomyelin; SPH: sphingosine; St: sterol; TG: triglyceride; WE: wax ester.

Regarding reproducibility, IPA-extracted lipids had a mean CV of 6.66%, whereas MTBE-extracted lipids had a mean CV of 5.65%. The lipids were classified into 4 groups based on their CV: CV below 5%, CV between 5% and 10%, CV between 10% and 20%, and CV between 20% and 30% (**Figure 7**).

For both solvents, approximately two-thirds of the extracted lipids had a CV below 5%. Specifically, 167 out of 262 lipids (63.7%) had a CV below 5% for IPA, and 181 out of 263 lipids (68.8%) had a CV below 5% for MTBE.

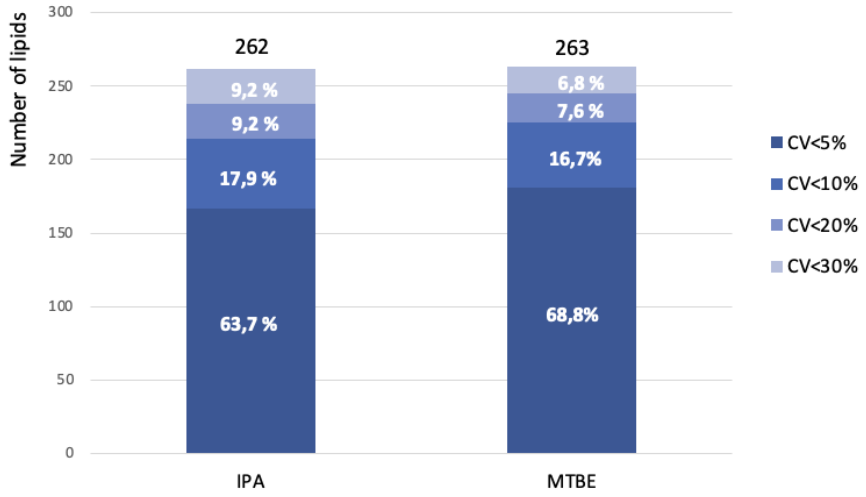

Figure 7. Distribution of lipids per coefficient of variation based on extraction solvent: IPA or MTBE. CV: coefficient of variation; IPA: isopropanol; MTBE: methyl tert-butyl ether.

Thus, we obtained equivalent numbers, nature, and reproducibility of lipids using both extraction solvents.

The relative quantity of lipids extracted using IPA was compared to that extracted using MTBE. The mean values for the sum of the peak intensities of the lipids extracted by IPA and MTBE are presented in **Figure 8**. The same procedure was applied to each lipid class common to both solvents (**Figure 9**).

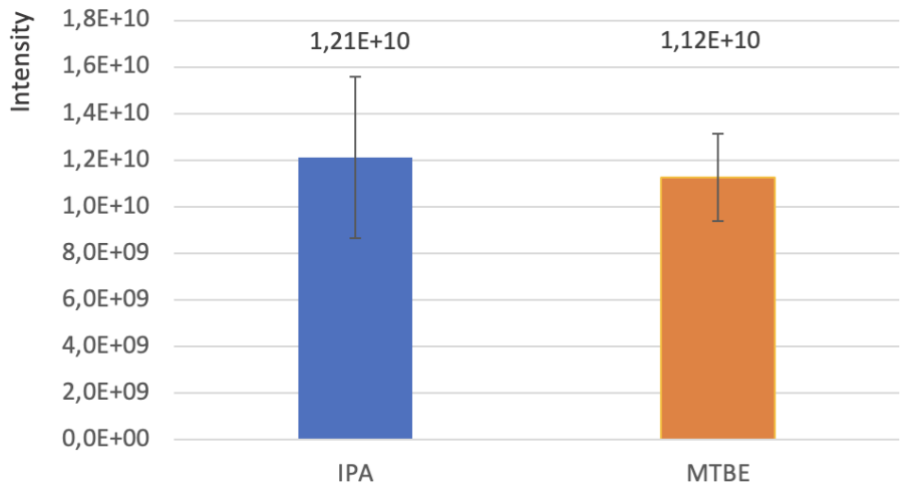

Figure 8. Means and standard deviations of peak intensities for lipids extracted with IPA and MTBE. IPA: isopropanol; MTBE: methyl tert-butyl ether.

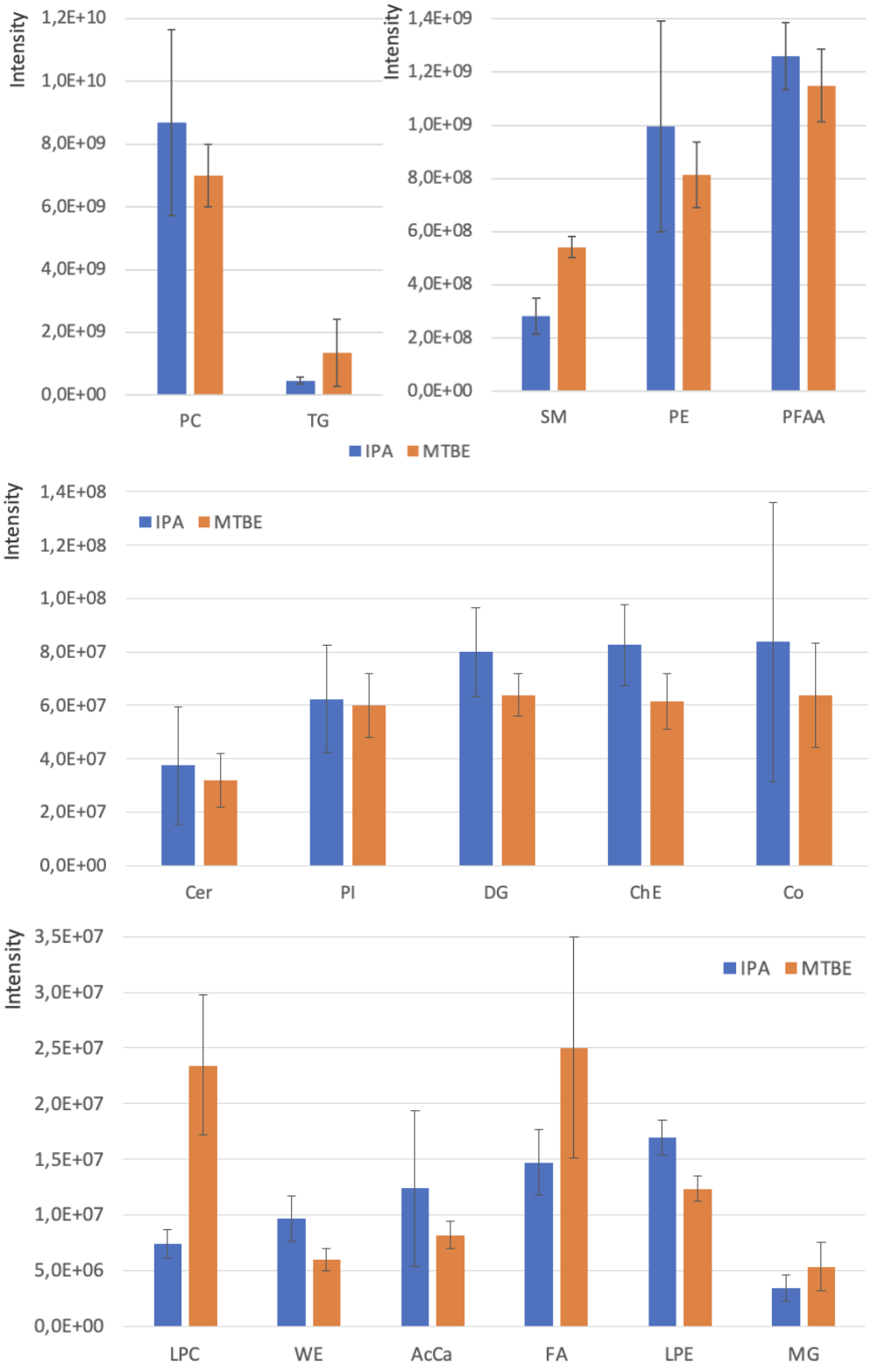

Figure 9. Means and standard deviations of peak intensities for lipids extracted with IPA and MTBE in the main classes common to both solvents. AcCA: fatty acyl carnitine; Cer: ceramide; ChE: cholesteryl ester; Co: Coenzyme Q; DG: diacylglycerol; FA: fatty acid; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; MG: monoacylglycerol; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PFAA: primary amide; PI: phosphatidylinositol; SM: sphingomyelin; WE: wax ester.

IPA extracted a greater quantity of lipids across all lipid classes, except for triglycerides (TG), sphingomyelins (SM), lysophosphatidylcholines (LPC), and fatty acids (FA) (**Figure 9**). However, the paired non-parametric univariate Wilcoxon test did not reveal any statistically significant differences in lipid intensities between the two solvents for any class.

In addition, when analyzing a large number of samples, it is important to consider the practical feasibility of the manipulations. Due to its miscibility with lipids, IPA produces a monophasic solution, making pipetting easier and reducing the risk of error. In contrast, biphasic solutions, such as those obtained with MTBE, can be a source of accidental sampling of the aqueous phase. The ease of manipulation makes IPA a suitable choice for lipid extraction.

As both solvents were equivalent in number, type, reproducibility, and amount of lipids extracted, we recommend IPA as solvent for lipid extraction from conjunctival IC specimens because of its simplicity. **Supplemental table 2** presents the lipid species of IC samples extracted with IPA and their CV for CQ (see page 52).

DISCUSSION

I. Variability of the metabolomic profile of successive IC specimens of different ocular sites from the same eye

In this study, we collected four IC specimens per eye to enhance the robustness of our methodological development while minimizing the number of subjects. The filter papers were applied to the superior bulbar conjunctiva, an area chosen for its protection by the eyelid, which minimizes the ocular surface reactions associated with exposure to environmental factors, thus increasing the reproducibility of sampling (24).

Significant variability was observed in the intensities of metabolite peaks obtained from 4 successive IC specimens from the same eye. This variability may be due to the physiologically heterogenous distribution of ocular surface cells along the conjunctiva. For example, goblet cells are absent from the limbus and gradually increase towards the fornix, and dendritic cells are more abundant in the supero-nasal quadrant (1). Furthermore, repeated application to the same area of the conjunctiva results in the collection of deeper cells from the basal layer of the conjunctival epithelium (24). It is important to note that the origin of the cells from which the metabolites are extracted (goblet, epithelial or inflammatory cells) cannot be identified. Thus, sampling location may add variability to the results.

Previous studies have shown that the quantity of ocular surface cells obtained per IC sample can vary, from 2.10^5 to 10^6 and from 2 up to 5 layers of epithelial cells, depending on the sampling method, including the duration of filter application and the amount of pressure applied (24). However, in our study, filters were applied to the conjunctival surface without exerting any pressure, and all samples were taken by the same operator to limit the variability associated with sampling. Moreover, minimal variations between samples due to pre-analytical conditions (sampling, sample preparation) can be balanced by normalizing peaks to the total area during the data processing stage.

In clinical practice, it is important to consider this variability, therefore, in the CATARACTOMIQUE study, it is essential to perform preoperative and postoperative IC specimens on the same location of the bulbar conjunctiva.

II. No statistically significant difference in the metabolic profile of the first 2 supra-temporal IC specimens

The absence of a statistically significant difference in peak intensities for 90% of the metabolites between the first two IC specimens is a noteworthy finding. The first two IC specimens were comparable, so we pooled them to establish our protocol for selecting the solvent for lipid extraction (IPA or MTBE), which increased the signal volume.

In the CATARACTOMIQUE study, two IC specimens per eye will be collected from the eye undergoing surgery, one for metabolomic analysis and one for lipidomic analysis. The fold change analysis of the 10% of metabolites whose peak intensities differed significantly between the two IC raises the question of which IC specimen should be used for metabolomic analysis and which for lipidomic analysis. In the left eyes, 12 of the 26 metabolites that varied between A' and B' were lipids detected by the HILIC column (11 phospholipids and 1 sphingomyelin), and all these lipids were more prominent in the second IC specimen (B'). This finding suggests that the second IC specimen should be used for lipidomic analysis. However, this trend was not observed in the right eyes, and needs to be confirmed by lipidomic analysis on a C18 column.

III. Inter-eye comparability of the metabo-lipidome: an essential requirement for selecting the extraction solvent

The variability of the 4 successive IC specimens led us to compare IC specimens of the same rank 2 by 2. As the compounds detected in both eyes were the same, the comparison focused on the peak intensities of these common metabolites and found no statistically significant difference. Indeed, during the processing of the raw mass spectrometer data, the metabolite detection algorithm retained a compound if it was present in both the right and left eyes of the 10 subjects, and if it was consistently present in all QC samples. It is therefore possible that metabolites present in one eye out of two, or in a few successive IC specimens out of four, or even in a few subjects, were not retained and analyzed. However, these excluded metabolites would be variable, and to develop a method as part of a biomarker discovery strategy, we need stable and robust metabolites that are reproducible across samples.

When analyzing human metabolomics, inter-individual variability can be caused by diet, environment, and genetic factors. This variability is not known in the context of metabo-lipidomic analysis of IC specimens and should be further investigated. Thanks to the comparability of the inter-eye conjunctival metabo-lipidome, inter-individual variability did not represent a confusion bias in our method for extraction solvent selection, as we used perfect controls represented by the fellow eye. We compared the compounds extracted from the right eye using one solvent to those extracted from the left eye using another solvent in the same subject. In this way, any differences in the number, reproducibility, or quantity of compounds obtained will be attributed to the tested solvent and not to the sample's laterality or subject variability.

IV. Potential for greater metabolic coverage of the ocular surface compared to tears

To date, all studies of the ocular surface's metabolomics have been conducted using tear samples (2). By comparing the metabolome of superficial conjunctival cells collected by IC specimens with tears under physiological conditions, we have demonstrated their complementarity. Specific metabolites were detected exclusively in IC specimens, and a greater number of metabolic pathways were significantly expressed in IC specimens. This is likely due to the intracellular metabolism of conjunctival cells, as opposed to the cell-free tear film. The presence of a metabolism specific to IC samples could promote the search for specific biomarkers that are not present in tears. Ultimately, a comprehensive biological analysis of the ocular surface should integrate both matrices.

However, it is important to note that the library of standard compounds used for targeted identification of the metabolites detected by the mass spectrometer was updated and expanded between the work carried out by Catanese *et al.* and our study (21). Therefore, the total number of metabolites detected in each matrix cannot be accurately compared. Furthermore, because of the semi-quantitative nature of the metabolomic analysis, it is not possible to compare the absolute quantities of metabolites obtained from separate analyses.

V. Determination of compounds extraction protocol

1. Considerations for selecting an extraction solvent for compounds

The omics approach starts with collecting, storing, and preparing samples, followed by analyzing them, and ends with processing data using bioinformatics tools to identify and quantify compounds. Each step in this workflow is crucial to transfer laboratory knowledge into clinical practice. However, sample preparation, especially the extraction of compounds, is particularly important, as it can have a strong impact on the sample composition. In the case of conjunctival IC specimens, extraction involves accessing the intracellular content of conjunctival cells adhering to the micropores of the solid matrix of the PES filter paper. Additionally, the process involves precipitating the proteins before analysis. To increase the likelihood of identifying biomarkers in future clinical studies, we aimed to select extraction solvents that would allow for the detection of the largest possible number of metabolites with varying physicochemical properties in a reproducible manner. Additionally, we aimed to choose solvents that were simple and time efficient.

2. Superiority of IPA extraction for lipidomics

Isopropanol and MTBE were chosen as candidate solvents due to their effectiveness in separating lipids from polar metabolites and precipitating proteins (27,28). Our team routinely uses them for lipid extraction from various matrices. The Folch protocol, which has been shown to be superior to MTBE for lipid extraction from tears (19), and the Bligh and Dyer method which is also widely used (23) were not tested in this study due to the presence of chloroform, which dissolves PES. As the number, nature, reproducibility, and amount of lipids extracted were comparable between IPA and MTBE, we chose IPA because of its single-phase nature, which reduces the risk of sampling errors during pipetting. Indeed, with MTBE, the collection of the lipid fraction requires a careful pipetting of the lower lipidic phase.

With 63.7% and 68.8% of lipids having a CV of less than 5% for IPA and MTBE respectively, the CV obtained were very low. However, it is important to note that the solid nature of the IC specimens limits the possibility of first forming a pool of samples that can be aliquoted into several replicates before the addition of extraction solvents, as was done for tears for the calculation of the CV (21). For IC samples, the QC pool can only be created after the

compounds have been extracted using a solvent to obtain a liquid matrix. The CV for QC were obtained by reinjecting this QC pool at different times during the analysis. This only measures the instrumental variability linked to the spectrometer and not the variability linked to the extraction process itself. This supports the selection of a solvent that reduces the risk of variability during manual sample manipulation, such as IPA.

VI. An innovative biomarker research strategy in ocular surface pathologies

The physiopathology of ocular surface diseases is not fully understood due to their multifactorial nature, and treatments remain unsatisfactory. Tear fluid studies in DED have identified over-expression of pathways related to the inflammatory response, the complement and coagulation cascade, glycolysis and gluconeogenesis, and amino acid metabolism (29). The pathophysiological mechanisms of DED include tear film hyperosmolarity and instability, cell apoptosis, ocular surface inflammation, and goblet cell loss, which create a vicious biological cycle (30). Lipids can contribute to instability of the tear film through the release of lipases by conjunctival microbial flora that has been altered. In the context of post-operative cataract surgery, factors that may contribute to DED include trauma to the corneal nerves, phototoxicity from the operating microscope, toxicity from anesthetics, antiseptics, and post-operative eye drops (15). These factors may constitute a gateway to the biological vicious circle, but DED may evolve independently of its causal factor, even once the latter has disappeared. This highlights the importance of early diagnosis of a latent inflammatory state which can be exacerbated by cataract surgery, by identifying predictive factors in the CATARACTOMIQUE study.

To our knowledge, only one study published in 2020 conducted a metabolomic analysis of IC specimens using UHPLC-MS/MS to identify diagnostic biomarkers for ocular mucous membrane pemphigoid (MMP) in a case-control study (n=16 patients, resulting in 32 IC specimens) (31). This study revealed altered levels of signaling lipid mediators associated with ocular MMP onset and progression, and identified oxylipins, lysophospholipids, fatty acids, and endocannabinoids as potential diagnostic biomarker candidates linked to inflammatory processes. The authors conducted a targeted lipidomic analysis with a specific focus on identifying signaling lipid mediators, rather than a metabolomic analysis. They conclude on the necessity to expand their method to a larger selection of metabolites to offer a broader knowledge of the ocular surface at the metabolite level: this formed the background to our study.

VII. Limitations and perspectives

The median age of the subjects in our study was 23, and these results may not apply to CATARACTOMIQUE patients, who must be over 50. Then, the impact of anesthetic drops, used to reduce patient discomfort, on the metabo-lipidome of conjunctival cells is unknown and should be further investigated. Although some authors do not use anesthesia for conjunctival IC (31), we used local anesthesia due to the multiplicity of samples in our study (4 double-sided IC per eye). Furthermore, if the IC technique is performed without anesthesia, patients may tend to close or move their eyes, making it more difficult for the examiner to collect accurate data. This could result in anomalous harvesting and a loss of reproducibility, especially for the final IC specimens.

Metabolomics provides a biochemical snapshot of the cells at a precise point in the disease course. Sample stability during storage at -80°C in an Eppendorf tube should be evaluated, especially to cover the expected timeframe from sample collection to analysis in the CATARACTOMIQUE study, which is approximately one year. Another perspective would be to evaluate the intra-individual variability over time of the IC specimens metabo-lipidomic profile. Furthermore, this variability could be assessed at various time points during a single day. Authors have reported a decrease in the number of cells collected per IC specimen at the end of the day, which is attributed to neutrophils present upon waking (32). The evaluation of inter-operator variability in the sampling technique should also be considered. Finally, it may be worth considering the use of semi-automated sampling methods such as Eyeprim (OPIA Technologies SAS, Paris, France) to standardize membrane placement, pressure, and handling when performing IC with multiple operators, and comparing it to our conventional IC method (33).

CONCLUSION

This study confirms the feasibility of metabo-lipidomics on IC specimens and provides the first literature description of the metabo-lipidomic profile of conjunctival cells collected by IC from healthy subjects. Our targeted approach, using an in-house database, robustly detected 211 metabolites associated with 9 significant metabolic pathways and 262 lipids from 24 lipid classes. Inter-eye metabolome comparability of conjunctival cells was established. The use of successive IC sampling should be considered in clinical practice as it may introduce pre-analytical variability; however, the first 2 successive supero-temporal IC specimens were comparable. IPA should be prefered for lipidomics extraction. Metabo-lipidomic analysis of IC specimens compared 132 specific metabolites to tears, thus providing an innovative and promising approach for identifying biomarkers in ocular surface diseases. In addition, standardized protocols for sampling, storage, preparation of samples, and analysis are necessary to obtain reliable and comparable results between different research teams. This study provides a simple and validated workflow that limits pre-analytical and analytical variability in order to transfer findings from bench to bedside.

REFERENCES

1. Pisella P, Baudouin C, Hoang-Xuan T. Rapport SFO 2015: surface oculaire. 2015;
2. Khanna RK, Catanese S, Emond P, Corcia P, Blasco H, Pisella PJ. Metabolomics and lipidomics approaches in human tears: A systematic review. Surv Ophthalmol. 2022;67(4):1229-43.
3. Brignole F, Pisella PJ, Goldschild M, De Saint Jean M, Goguel A, Baudouin C. Flow cytometric analysis of inflammatory markers in conjunctival epithelial cells of patients with dry eyes. Invest Ophthalmol Vis Sci. mai 2000;41(6):1356-63.
4. Baudouin C, Bourcier T, Brignole F, Bertel F, Moldovan M, Goldschild M, et al. Correlation between tear IgE levels and HLA-DR expression by conjunctival cells in allergic and nonallergic chronic conjunctivitis. Graefe's Archive for Clinical and Experimental Ophthalmology. 15 nov 2000;238(11):900-4.
5. Pisella PJ, Brignole F, Debbasch C, Lozato PA, Creuzot-Garcher C, Bara J, et al. Flow cytometric analysis of conjunctival epithelium in ocular rosacea and keratoconjunctivitis sicca. Ophthalmology. oct 2000;107(10):1841-9.
6. Hagan S. Biomarkers of ocular surface disease using impression cytology. Biomark Med. déc 2017;11(12):1135-47.
7. Patti GJ, Yanes O, Siuzdak G. Innovation: Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol. 22 mars 2012;13(4):263-9.
8. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. Chem Soc Rev. 2011;40(1):387-426.
9. Griffiths WJ, Ogundare M, Williams CM, Wang Y. On the future of « omics »: lipidomics. J Inherit Metab Dis. juin 2011;34(3):583-92.
10. Tan SZ, Begley P, Mullard G, Hollywood KA, Bishop PN. Introduction to metabolomics and its applications in ophthalmology. Eye (Lond). juin 2016;30(6):773-83.
11. Sullivan BD, Crews LA, Messmer EM, Foulks GN, Nichols KK, Baenninger P, et al. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications. Acta Ophthalmologica. mars 2014;92(2):161-6.
12. Bigoteau M, Grammatico-Guillon L, Massot M, Baudet JM, Cook AR, Duroi Q, et al. Ambulatory surgery centers: possible solution to improve cataract healthcare in medical deserts. J Cataract Refract Surg. mars 2021;47(3):352-7.

13. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, et al. TFOS DEWS II Definition and Classification Report. The Ocular Surface. juill 2017;15(3):276-83.
14. Sayegh RR, Yu Y, Farrar JT, Kuklinski EJ, Shtein RM, Asbell PA, et al. Ocular Discomfort and Quality of Life Among Patients in the Dry Eye Assessment and Management Study. Cornea. juill 2021;40(7):869-76.
15. Naderi K, Gormley J, O'Brart D. Cataract surgery and dry eye disease: A review. Eur J Ophthalmol. sept 2020;30(5):840-55.
16. Sullivan BD, Crews LA, Sönmez B, de la Paz MF, Comert E, Charoenrook V, et al. Clinical Utility of Objective Tests for Dry Eye Disease: Variability Over Time and Implications for Clinical Trials and Disease Management. Cornea. sept 2012;31(9):1000-8.
17. Nichols KK, Mitchell GL, Zadnik K. The Repeatability of Clinical Measurements of Dry Eye: Cornea. avr 2004;23(3):272-85.
18. Starr CE, Gupta PK, Farid M, Beckman KA, Chan CC, Yeu E, et al. An algorithm for the preoperative diagnosis and treatment of ocular surface disorders. Journal of Cataract and Refractive Surgery. mai 2019;45(5):669-84.
19. Catanese S, Khanna RK, Lefevre A, Alarcan H, Pisella PJ, Emond P, et al. Validation of metabolomic and lipidomic analyses of human tears using ultra-high-performance liquid chromatography tandem mass spectrometry. Talanta. 1 févr 2023;253:123932.
20. Martias C, Baroukh N, Mavel S, Blasco H, Lefèvre A, Roch L, et al. Optimization of Sample Preparation for Metabolomics Exploration of Urine, Feces, Blood and Saliva in Humans Using Combined NMR and UHPLC-HRMS Platforms. Molecules. 6 juill 2021;26(14):4111.
21. Catanese S, Khanna RK, Lefevre A, Alarcan H, Pisella PJ, Emond P, et al. Validation of metabolomic and lipidomic analyses of human tears using ultra-high-performance liquid chromatography tandem mass spectrometry. Talanta. Under revision;
22. Bardou P, Mariette J, Escudié F, Djemiel C, Klopp C. jvenn: an interactive Venn diagram viewer. BMC Bioinformatics. déc 2014;15(1):293.
23. Beauclercq S, Nadal-Desbarats L, Germain K, Praud C, Emond P, Bihan-Duval EL, et al. Does lipidomic serum analysis support the assessment of digestive efficiency in chickens? Poultry Science. mars 2019;98(3):1425-31.
24. Brignole-Baudouin F, Ott AC, Warnet JM, Baudouin C. Flow cytometry in conjunctival impression cytology: a new tool for exploring ocular surface pathologies. Experimental Eye Research. mars 2004;78(3):473-81.
25. Labbé A, Brignole-Baudouin F, Baudouin C. Méthodes d'évaluation de la surface

oculaire dans les syndromes secs. Journal Français d'Ophtalmologie. janv 2007;30(1):76-97.

26. Calonge M, Diebold Y, Sáez V, Enríquez de Salamanca A, García-Vázquez C, Corrales RM, et al. Impression cytology of the ocular surface: a review. Exp Eye Res. mars 2004;78(3):457-72.

27. Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. Journal of Lipid Research. mai 2008;49(5):1137-46.

28. Acera A, Pereiro X, Abad-Garcia B, Rueda Y, Ruzafa N, Santiago C, et al. A simple and reproducible method for quantification of human tear lipids with ultrahigh-performance liquid chromatography-mass spectrometry. Mol Vis. 2019;25:934-48.

29. Chen X, Rao J, Zheng Z, Yu Y, Lou S, Liu L, et al. Integrated Tear Proteome and Metabolome Reveal Panels of Inflammatory-Related Molecules via Key Regulatory Pathways in Dry Eye Syndrome. J Proteome Res. 3 mai 2019;18(5):2321-30.

30. Baudouin C. Un nouveau schéma pour mieux comprendre les maladies de la surface oculaire. Journal Français d'Ophtalmologie. mars 2007;30(3):239-46.

31. Di Zazzo A, Yang W, Coassin M, Micera A, Antonini M, Piccinni F, et al. Signaling lipids as diagnostic biomarkers for ocular surface cicatrizing conjunctivitis. J Mol Med. mai 2020;98(5):751-60.

32. Singh R, Joseph A, Umapathy T, Tint NL, Dua HS. Impression cytology of the ocular surface. Br J Ophthalmol. déc 2005;89(12):1655-9.

33. López-Miguel A, Gutiérrez-Gutiérrez S, García-Vázquez C, Enríquez-de-Salamanca A. RNA Collection From Human Conjunctival Epithelial Cells Obtained With a New Device for Impression Cytology. Cornea. janv 2017;36(1):59-63.

SUPPLEMENTAL DATA

Supplemental table 1. Metabolic profiling of the 80 conjunctival impression cytology specimens extracted with methanol and analyzed with UHLC-HRMS. CV: coefficient of variation; C18 Mode +/-: C18 column and positive/negative electrospray ionization mode; HILIC: hydrophilic interaction liquid chromatography column in positive electrospray ionization mode; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PC: phosphatidylcholine; QC: quality control; rt: retention time; SM: sphingomyelin; UHPLC-HRMS: ultra-high-performance liquid chromatography coupled to ultra-high resolution mass spectrometry.

Mode	Metabolites	rt	CV QC
C18 Mode -	L-ASPARTIC ACID	0,80	2,21
C18 Mode +	1-AMINOCYCLOPROPANECARBOXYLIC ACID	0,80	2,41
HILIC	1-METHYLADENOSINE	1,97	1,59
C18 Mode +	1-METHYLHISTIDINE	0,72	0,59
C18 Mode +	3-4-HYDROXYPHENYL-PYRUVATE	12,02	21,60
HILIC	3-DEHYDROXYCARNITINE	9,19	9,01
C18 Mode -	3-HYDROXYPHENYLACETIC ACID	8,96	9,18
C18 Mode -	3-METHYGLUTARIC ACID	5,68	6,64
C18 Mode -	3-SULFINOALANINE	1,19	2,79
HILIC	4-GUANIDINOBUTANOIC ACID	5,20	3,35
HILIC	4-HYDROXY-3-METHOXYPHENYLGLYCOL	1,26	24,21
C18 Mode -	4-HYDROXYPROLINE	0,82	1,64
C18 Mode +	5-AMINOLEVULINIC ACID	0,81	14,00
C18 Mode +	5'-METHYLTHIOADENOSINE	6,39	20,42
C18 Mode -	ADENINE	2,55	1,86
C18 Mode +	ADENOSINE	2,54	16,34
C18 Mode +	ADENOSINE MONOPHOSPHATE	1,43	3,60
C18 Mode -	ADP-GLUCOSE	3,01	5,21
C18 Mode -	ALLANTOIN	0,86	1,58
C18 Mode -	ALPHA-GLUCOSE	0,83	3,35
C18 Mode +	AMINOADIPIC ACID	0,86	5,12
HILIC	ANSERINE	17,50	4,56
C18 Mode -	AZELAIC ACID	9,74	3,23
HILIC	BETA-ALANINE	9,10	7,42
HILIC	BETAINE	9,71	15,13
HILIC	BUTYRYLCARNITINE	7,82	0,89
HILIC	C14-CARNITINE	5,13	7,20
HILIC	C18-CARNITINE	4,68	9,29
HILIC	C5-CARNITINE	7,28	15,71
HILIC	C8-CARNITINE	6,05	2,15
HILIC	CAPRYLIC ACID	1,30	3,47

HILIC	CHOLESTERYL ACETATE	1,10	1,03
C18 Mode -	CILIATINE	0,80	2,13
C18 Mode +	CINNAMIC ACID	9,76	1,80
HILIC	CITICOLINE	18,40	5,20
HILIC	CITRULLINE	10,52	2,18
C18 Mode -	CREATINE	0,83	5,21
C18 Mode +	CREATINE PHOSPHATE	0,98	9,93
C18 Mode +	CREATININE	0,83	1,07
HILIC	CYTIDINE	3,27	2,09
HILIC	CYTOSINE	3,27	2,43
C18 Mode +	DEOXYCHOLATE	14,82	1,83
C18 Mode +	DIAMINOPIMELIC ACID	0,70	3,62
HILIC	DIETHANOLAMINE	6,64	1,83
HILIC	DL-GLUTAMATE	8,97	3,80
C18 Mode +	EPINEPHRINE	0,76	1,18
C18 Mode +	ETHANOLAMINE PHOSPHATE	0,79	5,33
C18 Mode -	FUMARATE	1,58	3,00
C18 Mode -	GALACTARIC ACID	0,98	1,50
HILIC	GAMMA-LINOLENIC ACID	1,18	0,99
C18 Mode -	GLUCONIC ACID	0,86	4,26
C18 Mode -	GLUCOSAMINE 6-PHOSPHATE	0,97	3,68
C18 Mode +	GLUCOSE 1-PHOSPHATE	0,99	5,54
C18 Mode -	GLUCOSE 6-PHOSPHATE	0,99	4,10
HILIC	GLUCURONOLACTONE	1,87	1,99
C18 Mode +	GLYCERALDEHYDE 3-PHOSPHATE DIETHYL ACETAL	10,77	1,19
C18 Mode -	GLYCERATE	0,93	2,84
HILIC	GLYCEROPHOSPHOCHOLINE	15,61	7,32
C18 Mode +	GLYCINE	0,77	8,89
HILIC	GUANINE	2,66	5,25
HILIC	GUANOSINE	2,68	4,86
C18 Mode +	GUANOSINE 5'-MONOPHOSPHATE	1,76	8,63
C18 Mode -	HIPPURIC ACID	7,03	1,81
C18 Mode -	HOMOGENTISATE	5,68	2,32
C18 Mode -	HYDROXYPHENYLLACTIC ACID	5,99	4,87
C18 Mode +	HYPOXANTHINE	1,41	5,05
C18 Mode -	INDOXYL SULFATE	7,61	5,20
C18 Mode -	INOSINE	3,01	11,58
C18 Mode -	INOSINE 5'-MONOPHOSPHATE	2,09	20,60
C18 Mode -	ISOCITRIC ACID	0,99	14,57
HILIC	L-ACETYLCARNITINE	9,17	1,48
C18 Mode +	L-ALANINE	0,79	3,08
C18 Mode -	L-ARABITOL	0,85	5,84
C18 Mode +	L-ARGININE	0,72	15,58
C18 Mode +	L-ASPARAGINE	0,78	8,27

HILIC	L-CARNITINE	11,38	3,93
C18 Mode -	L-CYSTINE	0,77	2,34
HILIC	L-DOPA	1,41	3,06
C18 Mode +	L-GLUTAMINE	0,79	2,51
C18 Mode +	L-HISTIDINE	0,72	9,58
C18 Mode -	L-HOMOSERINE	0,80	1,60
C18 Mode +	L-LYSINE	0,69	2,52
C18 Mode -	L-METHIONINE	1,15	3,29
C18 Mode +	L-PHENYLALANINE	3,34	6,34
HILIC	L-PROLINE	8,53	2,49
HILIC	L-SERINE	8,81	9,38
C18 Mode +	L-THREONINE	0,80	7,22
C18 Mode -	L-TRYPTOPHAN	5,27	7,50
C18 Mode -	L-TYROSINE	1,45	2,46
C18 Mode -	LACTATE	1,25	10,04
HILIC	LAUROYLCARNITINE	5,40	4,44
C18 Mode -	LEUCINE	1,65	8,50
C18 Mode +	LEUCINE ISOLEUCINE NORLEUCINE	1,65	5,29
HILIC	LYSOPC(14_0)	9,90	10,54
HILIC	LYSOPC(16_0)	9,44	8,75
HILIC	LYSOPC(16_1)	9,52	1,95
HILIC	LYSOPC(18_0)	9,24	2,88
HILIC	LYSOPC(18_1)	9,30	10,15
HILIC	LYSOPC(18_2)	9,40	5,87
HILIC	LYSOPC(19_1)	8,30	15,99
HILIC	LYSOPC(20_2)	9,22	43,57
HILIC	LYSOPC(20_3)	9,26	5,63
HILIC	LYSOPC(20_4)	9,24	18,42
HILIC	LYSOPC(20_5)	9,33	5,34
HILIC	LYSOPE(16_0)	6,48	5,99
HILIC	LYSOPE(18_0)	6,33	6,87
HILIC	LYSOPE(18_1)	6,37	11,85
HILIC	LYSOPE(18_2)	6,44	6,56
HILIC	LYSOPE(20_5)	6,37	19,58
HILIC	LYSOPI(18_0)	5,56	11,29
C18 Mode +	LYXOSE	1,22	11,12
C18 Mode -	MALATE	1,07	21,90
C18 Mode -	MANNITOL	0,84	76,17
HILIC	METHYL JASMONATE	1,22	24,81
HILIC	METHYLGUANIDINE	4,51	25,77
C18 Mode +	METHYLMALONIC ACID	2,98	6,73
HILIC	MONO-METHYL GLUTARATE	1,09	15,35
C18 Mode -	N-ACETYL-L-ASPARTIC ACID	1,34	6,66
C18 Mode -	N-ACETYL-L-GLUTAMIC ACID	1,76	19,34
C18 Mode -	N-ACETYL-L-METHIONINE	6,40	3,11

C18 Mode -	N-ACETYL-L-PHENYLALANINE	8,86	5,13
C18 Mode -	N-ACETYLNEURAMINATE	1,00	4,91
C18 Mode -	N-ACETYLSERINE	0,81	5,64
C18 Mode +	N-ACETYLSEROTONIN	8,81	11,67
C18 Mode -	N6-(DELTA2-ISOPENTENYL)-ADENINE	9,03	7,92
HILIC	N6,N6,N6-TRIMETHYL-L-LYSINE	17,87	9,96
C18 Mode +	NIACINAMIDE	1,42	27,00
C18 Mode +	OMEGA-HYDROXYDODECANOIC ACID	11,45	21,54
C18 Mode +	OPHTHALMIC ACID	1,55	8,30
C18 Mode +	ORNITHINE	0,69	2,89
C18 Mode -	OXOGLUTARIC ACID	1,36	6,20
C18 Mode -	PANTOTHENIC ACID	5,62	4,33
HILIC	PC(25_0)	7,93	6,40
HILIC	PC(29_0)	7,18	5,23
HILIC	PC(30_0)	7,14	7,36
HILIC	PC(31_0)	7,11	6,40
HILIC	PC(32_0)	7,08	18,23
HILIC	PC(32_1)	7,03	6,22
HILIC	PC(32_2)	7,04	11,87
HILIC	PC(33_0)	7,06	8,89
HILIC	PC(33_1)	6,99	8,16
HILIC	PC(33_2)	6,99	15,78
HILIC	PC(34_1)	6,95	12,99
HILIC	PC(34_2)	6,95	66,37
HILIC	PC(34_3)	6,94	11,82
HILIC	PC(34_4)	6,86	34,04
HILIC	PC(35_1)	6,93	13,72
HILIC	PC(35_2)	6,92	41,93
HILIC	PC(35_3)	6,90	11,06
HILIC	PC(35_4)	6,81	23,54
HILIC	PC(36_1)	6,90	6,95
HILIC	PC(36_2)	6,88	7,03
HILIC	PC(36_3)	6,86	9,59
HILIC	PC(36_4)	6,81	13,88
HILIC	PC(36_5)	6,79	14,81
HILIC	PC(37_5)	6,74	16,06
HILIC	PC(38_2)	6,84	26,89
HILIC	PC(38_3)	6,81	7,25
HILIC	PC(38_6)	6,72	12,48
HILIC	PC(39_6)	6,67	5,15
HILIC	PC(40_5)	6,68	5,19
HILIC	PC(40_7)	6,62	9,72
HILIC	PC(40_9)	6,70	24,19
HILIC	PC(O-12_0__2_0)	9,67	7,68
HILIC	PE(16_0__18_2)	3,58	8,94

HILIC	PE(18_1__18_1)	3,57	25,58
C18 Mode -	PHOSPHOCREATINE	0,98	7,28
HILIC	PHOSPHORYLCHOLINE	18,06	8,30
HILIC	PROPIONYLCARNITINE	8,44	7,07
C18 Mode +	PYRIDOXAMINE	0,70	6,01
C18 Mode -	PYROGLUTAMIC ACID	1,61	27,53
C18 Mode -	QUINATE	0,94	7,97
C18 Mode +	RAC-GLYCEROL 1-MYRISTATE	13,27	10,51
C18 Mode +	RIBOFLAVIN	8,25	12,50
C18 Mode -	RIBOSE 5-PHOSPHATE	1,02	18,37
HILIC	SM(30_1)	8,74	7,38
HILIC	SM(32_0)	8,61	10,20
HILIC	SM(32_1)	8,61	27,06
HILIC	SM(32_2)	8,64	16,31
HILIC	SM(33_1)	8,55	15,42
HILIC	SM(34_0)	8,50	7,35
HILIC	SM(34_1)	8,50	23,29
HILIC	SM(34_2)	8,51	21,03
HILIC	SM(35_1)	8,46	14,58
HILIC	SM(36_1)	8,41	6,80
HILIC	SM(36_2)	8,40	8,07
HILIC	SM(38_1)	8,34	6,93
HILIC	SM(38_2)	8,31	5,67
HILIC	SM(39_1)	8,32	15,92
HILIC	SM(40_2)	8,24	4,29
HILIC	SM(40_3)	8,24	7,91
HILIC	SM(42_2)	8,22	7,13
HILIC	SM(42_3)	8,19	5,50
HILIC	SORBATE	1,21	10,79
HILIC	SPERMIDINE	17,79	12,62
C18 Mode -	SUBERIC ACID	8,93	6,92
C18 Mode -	SUCCINIC ACID	1,64	20,48
HILIC	TAURINE	4,35	7,16
HILIC	THEOBROMINE	1,53	22,77
C18 Mode -	THEOPHYLLINE	6,59	6,64
HILIC	TRIGONELLINE	9,68	4,82
HILIC	TRIMETHYLAMINE	7,78	8,49
HILIC	URACIL	1,62	8,06
C18 Mode +	URATE	1,31	35,63
C18 Mode -	URIDINE	1,84	20,22
C18 Mode -	URIDINE 5'-DIPHOSPHO-N-ACETYLGLUCOSAMINE	2,49	8,44
C18 Mode -	URIDINE 5'-DIPHOSPHOGLUCOSE	2,15	7,25
C18 Mode -	URIDINE 5'-MONOPHOSPHATE	1,59	5,72
C18 Mode +	UROCANIC ACID	0,94	6,40
HILIC	VITAMIN K1	1,29	10,55
C18 Mode -	XANTHINE	1,63	91,95

Supplemental table 2. Lipidic profiling of the 6 conjunctival impression cytology specimens extracted with isopropanol and analyzed with UHLC-HRMS. AcCA: fatty acyl carnitine; BisMePA: bis-methyl phosphatidic acid; Cer: ceramide; ChE: cholesteryl ester; CmE: sterol ester; Co: Coenzyme Q; CV: coefficient of variation; DG: diacylglycerol; FA: fatty acid, Hex1Cer: monoexosylceramide; LBPA: lysobisphosphatidic acid; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; m/z: mass-to-charge ratio MePC: methylphosphatidylcholine; MG: monoacylglycerol; PA: phosphatidic acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PFAA: primary amide; PG: phosphatidylglycerol; PI: phosphatidylinositol; QC: quality control; rt: retention time SM: sphingomyelin; SPH: sphingosine; St: sterol; TG: triglyceride; WE: wax ester.

Lipids	m/z	rt	CV QC
AcCa(16:0)	400,342712	2,104907	2,55
AcCa(18:1)	426,358337	2,22065981	3,26
BisMePA(39:7)	778,539063	8,80311382	7,10
BisMePA(40:9)	788,523743	7,22786115	10,44
Cer(d18:1_9:0)	440,410706	3,36854894	25,13
Cer(d18:0_16:0)	540,535767	10,7504689	2,96
Cer(d18:1_16:0)	538,520325	10,1277359	1,64
Cer(d18:2_16:0)	536,5047	8,86079352	3,81
Cer(d18:1_18:0)	566,551758	11,7156178	1,75
Cer(d18:2_18:0)	564,536255	10,4850729	10,29
Cer(d18:1_22:0)	622,614075	14,4829912	3,09
Cer(d18:1_23:0)	636,630127	15,1644142	3,41
Cer(d18:1_24:0)	694,637634	15,8983507	1,70
Cer(d18:1_24:2)	646,61438	13,4643792	2,85
Cer(d18:1_26:1)	676,661194	15,8161598	8,84
ChE(16:0)	642,619263	22,2172437	7,97
ChE(16:1)	640,603699	21,6190752	4,62
ChE(18:0)	670,650635	22,6424585	1,08
ChE(18:1)	668,634583	22,2488769	1,64
ChE(18:3)	664,603821	21,0830318	6,64
ChE(20:4)	690,61908	21,4002747	2,90
ChE(20:5)	688,60376	20,5547358	4,30
CmE(18:2)	680,635254	21,9824582	23,63
Co(Q10)	880,718262	19,7491422	1,50
Co(Q9)	812,65625	17,5362175	6,45
DG(14:0_18:1)	584,525909	11,8040126	16,36
DG(16:0_18:0)	614,572754	14,288538	9,12
DG(16:0_18:1)	612,557068	13,1744673	2,00
DG(16:0_18:2)	610,541382	12,1465918	2,25
DG(18:1_18:0)	640,588318	13,1740382	9,59
DG(36:2)-1	638,572601	13,3560412	18,32
DG(36:2)-2	638,572754	13,5103247	23,24
DG(18:2_18:2)	634,541504	11,2546068	1,77
DG(18:0_20:3)	664,58844	13,8644707	1,28

DG(18:1_20:4)	660,557312	12,1395816	11,55
DG(16:0_22:6)	658,541443	11,5710985	3,51
DG(20:1_20:4)	688,588989	13,4188374	13,54
DG(18:0_22:6)	686,572937	12,9851416	0,77
DG(18:1_22:6)	684,557343	11,7649161	15,54
DG(P-21:4)	408,308868	3,68065608	1,66
FA(16:0)	255,233139	4,23296267	1,72
FA(18:0)	283,264709	5,43122169	3,43
FA(18:1)	281,249207	4,42591466	3,27
FA(18:2)	279,23349	3,6955082	3,83
FA(20:3)	305,249451	3,99856423	3,73
FA(20:4)	303,23349	3,56452747	1,04
LBPA(16:0_18:1)	747,520386	7,74435132	1,00
LPC(16:0)	540,332275	2,0755512	1,33
LPC(16:1)	494,324982	1,74036999	3,56
LPC(17:0)	510,356506	2,38006475	9,21
LPC(18:0)	568,363678	2,78706491	1,80
LPC(18:1)	566,3479	2,18490887	3,87
LPC(20:2)	548,372009	2,32776581	23,70
LPC(20:4)	544,34137	1,744914	1,73
LPC(22:6)	568,341278	1,7019129	26,90
LPC(P-16:0)	480,345718	2,31575489	5,56
LPE(16:0)	454,293518	2,1446492	2,82
LPE(18:0)	480,310974	2,87857777	1,94
LPE(18:1)	478,295258	2,25437866	1,61
LPE(20:4)	502,293701	1,79083937	2,61
LPE(O-15:1)	424,28299	3,68065608	2,91
MePC(34:3)	771,575256	9,19583377	5,24
MG(18:1)	374,326889	3,55118913	23,28
PA(O-19:3_18:1)	714,544128	9,91025756	4,42
PC(25:0CHO)	650,440125	2,74829642	16,36
PC(27:0CHO)	678,471497	3,62268405	19,49
PC(30:0)	706,539001	7,53608102	1,02
PC(25:1_5:0)	704,52301	8,93287304	7,94
PC(31:0)	720,554382	8,22338197	4,35
PC(32:0)	734,570251	8,97479059	0,93
PC(16:0_16:0)	778,56189	9,01639108	1,67
PC(32:1)	732,554443	7,81649449	1,72
PC(14:0_18:1)	776,54657	7,86096562	0,63
PC(32:2)	730,539001	6,75566721	3,79
PC(33:1)	746,570068	8,4744898	2,81
PC(33:2)	744,554565	7,41852037	1,78
PC(15:2_18:0)	744,555115	10,1008761	2,99
PC(15:0_18:2)	788,546753	7,46044678	2,14
PC(33:3)	742,539307	8,78362701	5,47

PC(34:0)	762,60144	10,5243526	3,05
PC(16:0_18:0)	806,593292	10,556565	2,94
PC(34:1)	760,585266	9,18207729	3,03
PC(16:0_18:1)	804,577087	9,22805748	0,74
PC(34:2)	758,569763	8,1034421	0,49
PC(34:4)	754,538635	9,14682642	3,99
PC(35:1)	774,601501	9,97895492	2,08
PC(17:0_18:1)	818,593323	10,0235584	6,55
PC(35:2)	772,585571	8,87152197	4,70
PC(17:0_18:2)	816,57782	8,9094163	2,38
PC(35:4)	768,554504	7,25708936	5,07
PC(35:5)	766,539001	8,6092443	1,50
PC(35:7)	762,506348	8,3878173	4,48
PC(36:1)	788,616821	10,7313512	3,65
PC(36:2)	786,600769	9,41012513	1,97
PC(36:2)	786,601135	9,61191876	2,92
PC(36:3)	784,585083	8,31280547	2,37
PC(18:1_18:2)	828,577393	8,39645678	6,73
PC(18:2_18:2)	826,561951	7,33149518	1,99
PC(16:0_20:4)	826,562317	7,99820506	1,27
PC(36:5)	780,554321	7,07495178	2,59
PC(37:2)	800,617432	10,1564385	1,75
PC(37:4)	796,585938	8,70078035	1,33
PC(37:5)	794,570862	10,0306727	4,68
PC(37:6)	792,554932	6,9617051	7,30
PC(15:1_22:6)	790,538452	8,27555163	4,64
PC(38:2)	814,63269	10,8640623	3,15
PC(18:0_20:3)	856,608643	10,1043839	2,15
PC(38:4)	810,600708	9,44191292	2,69
PC(38:5)	808,585083	8,19637171	2,04
PC(18:1_20:4)	852,577026	8,19356802	1,62
PC(18:2_20:4)	850,562317	7,17220195	8,48
PC(16:0_22:6)	850,562622	7,69470304	3,04
PC(38:7)	804,55249	7,95633571	6,53
PC(39:6)	820,587402	8,34650904	25,51
PC(40:5)	836,617004	9,54904432	8,33
PC(40:6)	834,601379	9,09980664	5,65
PC(18:0_22:6)	878,59317	9,12348574	6,43
PC(40:7)	832,585999	7,84935119	6,09
PC(18:1_22:6)	876,578278	7,89042447	24,06
PC(40:8)	830,570557	6,85364052	7,04
PC(42:10)	854,570679	6,62997263	8,77
PC(44:12)	878,574585	6,35709071	4,21
PC(O-32:1)	718,574707	9,73910686	2,40
PC(O-34:1)	746,606384	10,2110017	8,25

PC(O-37:8)	774,543884	8,98179676	5,87
PC(O-39:10)	798,541077	9,18746067	7,15
PC(O-17:1_22:6)	804,591522	11,8442776	4,42
PC(O-41:10)	826,572754	10,7257894	9,31
PC(P-15:2_20:4)	748,528351	8,23649867	0,77
PC(P-37:9)	770,510437	8,77438934	5,55
PE(16:0_18:1)	718,539063	9,67522789	3,59
PE(16:0_18:2)	716,523376	8,56253437	2,47
PE(35:2)	730,539368	9,32641125	6,95
PE(18:0_18:1)	746,570374	11,2179296	3,08
PE(18:0_18:2)	742,540588	10,1374308	1,60
PE(18:1_18:2)	740,525269	8,82311971	0,81
PE(18:2_18:2)	740,52356	7,69879209	6,86
PE(20:4_16:0)	740,523193	8,37986445	3,34
PE(36:5)	738,505249	8,56200757	6,41
PE(18:0_20:3)	770,570557	10,5338194	3,20
PE(20:4_18:0)	768,554443	9,92015343	1,31
PE(18:0_20:4)	766,540283	9,95964252	1,49
PE(16:0_22:5)	766,538879	8,95756122	27,29
PE(18:1_20:4)	764,524963	8,65246871	4,20
PE(16:0_22:6)	762,509094	8,09340535	1,27
PE(18:0_22:4)	796,586182	11,0103225	2,70
PE(20:1_20:4)	792,556091	10,0650025	2,28
PE(18:0_22:6)	792,553772	9,56849149	2,28
PE(18:1_22:6)	788,524902	8,31218186	0,76
PE(O-18:2_14:0)	674,51236	8,99835453	11,21
PE(O-16:1_18:1)	700,529968	10,5425112	4,29
PE(O-18:2_16:1)	700,528137	9,35182185	4,09
PE(O-18:1_18:1)	728,56189	12,0384778	2,86
PE(O-16:1_20:4)	722,514099	9,16905274	1,14
PE(O-16:1_22:4)	750,545868	10,3033457	20,43
PE(O-18:1_20:4)	750,545349	10,7384181	0,71
PE(O-18:2_20:4)	748,529724	9,37475586	6,91
PE(O-16:1_22:6)	746,514343	8,81548364	0,91
PE(O-20:1_20:4)	780,591003	12,2116784	6,97
PE(O-18:2_22:4)	776,552734	10,3661175	6,48
PE(O-18:1_22:6)	774,545898	10,3666324	3,22
PE(O-18:2_22:6)	772,530334	9,02500985	1,33
PE(P-16:0_18:1)	702,544189	10,5124151	2,79
PE(P-18:0_18:1)	730,5755	12,01316	6,92
PE(P-16:0_20:3)	726,544189	9,78144714	2,76
PE(P-16:0_20:4)	724,528076	9,14212147	3,35
PE(P-18:0_20:1)	758,607025	13,3094002	16,82
PE(P-18:0_20:3)	754,575623	11,3326681	8,48
PE(P-16:0_22:4)	752,56012	10,2725671	20,44

PE(P-18:0_20:4)	752,559448	10,7061833	3,44
PE(P-18:1_20:4)	750,544189	9,3392311	3,60
PE(P-16:0_22:6)	748,528137	8,77631743	3,94
PE(P-18:0_22:4)	780,59137	11,7799584	10,67
PE(P-18:0_22:6)	776,559814	10,3294385	2,36
PFAA(18:1)	282,279465	3,39254788	25,91
PFAA(20:1)	310,310699	4,32707363	1,19
PFAA(22:0)	340,357635	6,11340766	1,01
PFAA(22:1)	338,341797	5,44238292	0,95
PFAA(22:2)	336,326324	4,56894775	1,20
PFAA(24:1)	366,373474	6,81944919	0,40
PG(34:2)	764,544556	6,77141126	4,29
PG(22:6_22:6)	884,545685	4,91041788	22,15
PI(34:2)	852,560913	6,17565312	2,83
PI(16:0_18:2)	833,521179	6,2337288	0,48
PI(36:1)	882,607239	8,4299728	3,85
PI(36:2)	880,591858	7,44915601	1,69
PI(18:0_18:2)	861,551575	7,51250499	1,19
PI(16:0_20:4)	857,52124	6,12489821	2,35
PI(38:3)	906,607605	7,83539917	2,39
PI(20:4_18:0)	904,591675	7,31881849	3,00
PI(18:0_20:4)	885,551392	7,38677473	0,28
PI(38:6)	900,560669	5,81893185	2,01
PI(16:0_22:6)	881,521606	5,8791149	1,81
PI(40:6)	928,59198	7,03734782	3,11
PI(18:0_22:6)	909,552307	7,1056536	0,77
SM(d32:1)	675,544556	5,92402164	3,87
SM(d33:1)	689,56012	6,55955039	3,53
SM(d34:0)	705,591125	7,7799944	2,27
SM(d34:1)	747,567383	7,25454294	0,74
SM(d34:2)	701,56012	6,17800788	3,72
SM(d35:1)	717,590942	7,96206268	2,86
SM(d36:0)	733,622986	9,28823309	4,67
SM(d36:1)	775,598389	8,76538921	1,68
SM(d36:2)	729,59137	7,55624258	4,70
SM(d36:4)	725,557068	7,21497003	5,24
SM(d38:2)	757,623596	9,09699612	4,31
SM(d40:1)	787,669617	11,8493711	4,33
SM(d40:2)	785,654541	10,4085149	2,84
SM(d42:1)	815,700562	13,2514846	1,97
SM(d42:2)	857,677307	11,872953	2,59
SM(d42:3)	855,661072	10,6935427	2,41
SM(d44:5)	835,667328	11,8362477	10,68
SPH(d15:1)	258,243134	2,01005329	23,49
St()	413,378128	9,02346242	10,17
TG(14:0_10:0_12:0)	656,583221	15,2134377	20,53

TG(10:0_14:0_16:0)	712,646027	17,9355578	14,68
TG(12:0_14:0_16:0)	740,677399	19,3030065	17,28
TG(25:0_18:1)	752,678131	18,7579055	25,12
TG(14:0_16:0_16:0)	796,739746	21,5332612	20,67
TG(14:0_15:0_18:1)	808,739319	21,3415474	18,69
TG(14:0_16:0_18:0)	824,770752	22,0673825	3,25
TG(14:0_16:0_18:1)	822,755188	21,6086604	15,22
TG(16:0_16:0_17:0)	838,786743	22,2659426	17,16
TG(15:0_16:0_18:1)	836,770691	21,8860117	7,86
TG(15:0_16:1_18:1)	834,755432	21,4399454	10,40
TG(16:0_16:1_18:1)	848,770691	21,7067161	3,79
TG(16:1_16:1_18:1)	846,755676	21,0942782	12,47
TG(16:0_17:0_18:1)	864,802246	22,3038698	4,48
TG(16:0_17:1_18:1)	862,786682	21,9513115	2,84
TG(16:0_18:0_18:0)	880,833374	22,7421777	2,81
TG(16:0_18:0_18:1)	878,817444	22,4778002	30,19
TG(16:0_18:1_18:1)	876,801819	22,1590002	2,41
TG(16:0_18:1_18:2)	874,786194	21,7751603	2,51
TG(16:1_18:1_18:2)	872,770813	21,2607316	2,88
TG(17:0_18:0_18:1)	892,833801	22,6170514	6,67
TG(18:0_18:0_18:0)	908,864716	22,978528	2,97
TG(18:0_18:1_18:1)	904,833374	22,5134792	1,78
TG(26:1_10:1_18:1)	902,817383	22,202793	2,23
TG(18:1_18:1_18:2)	900,802185	21,8416505	1,62
TG(18:1_18:2_18:2)	898,786499	21,3508507	4,52
TG(16:0_16:0_22:6)	896,771027	21,3645262	5,87
TG(25:0_15:0_15:0)	922,880737	23,0749046	10,62
TG(18:0_18:1_20:1)	932,864929	22,7789051	3,02
TG(18:1_20:1_18:2)	928,83374	22,2826019	23,32
TG(29:3_7:0_20:2)	926,818237	21,3526194	5,75
TG(18:1_16:0_22:5)	924,801392	21,7762424	10,92
TG(16:0_18:1_22:6)	922,786682	21,4371512	23,74
TG(16:0_18:2_22:6)	920,771057	20,7368404	5,16
TG(25:0_16:0_16:0)	950,91217	23,2585531	5,96
TG(21:1_18:1_18:1)	944,865662	22,6875719	20,65
TG(26:0_16:0_16:0)	964,927979	23,3521792	28,11
TG(18:1_20:1_20:1)	958,880615	22,8009927	2,85
TG(38:0_20:4)	956,865112	21,8340981	27,67
TG(18:1_22:1_18:2)	956,865234	22,5848983	1,04
TG(18:0_18:1_22:4)	954,849548	22,3980547	23,36
TG(18:1_18:1_22:4)	952,834229	22,0794537	14,10
TG(18:1_18:1_22:6)	948,802673	21,5573549	5,68
TG(O-13:0_16:0_3:0)	586,541504	12,977476	6,76
TG(O-15:0_20:4_3:0)	662,572388	13,3246618	1,62
TG(P-23:6_21:1)	721,578125	16,3322936	8,69
WE(O-16:2)	270,242981	3,88644661	3,40
WE(O-18:1)	300,290009	4,22099065	2,01

Vu, le Directeur de Thèse

Raoul Kanav KHANNA

Vu, le Doyen
De la Faculté de Médecine de Tours
Tours, le

LECOLIER Nattie
59 pages – 10 tableaux – 9 figures

Résumé :
Introduction : Les pathologies de la surface oculaire se manifestent par des symptômes et signes cliniques non spécifiques. L'empreinte conjonctivale (EC) est une méthode de prélèvement cellulaire rapide et non-invasive ayant démontré son utilité dans le diagnostic et la compréhension des mécanismes physiopathologiques de ces pathologies. L'approche métabo-lipidomique appliquée aux EC pourrait permettre l'identification de biomarqueurs mais nécessite une standardisation des procédures pré-analytiques et analytiques. L'objectif de cette étude était de valider les étapes pré-analytiques et analytiques de l'analyse métabo-lipidomique d'EC par chromatographie liquide ultra-haute-performance couplée à la spectrométrie de masse haute-résolution.
Matériel et méthodes : Quatre EC ont été réalisées successivement sur les yeux droits et gauches de 20 sujets sains, soit 160 empreintes. Trois protocoles d'extraction des métabolites (méthanol (MeOH), MeOH/eau et acétonitrile) et 2 protocoles d'extraction des lipides (méthyl tert-butyl éther et isopropanol (IPA)) ont été testés. Les métabolites identifiés ont été comparés ceux retrouvés dans les larmes humaines.
Résultats : Nous avons identifié 211 métabolites impliqués dans 9 voies métaboliques. Bien qu'il existe une variabilité importante des 4 empreintes réalisées sur le même œil, les deux premières étaient comparables. Le métabolome conjonctival des deux yeux était comparable. La comparaison avec les 137 métabolites identifiés dans les larmes a montré 79 métabolites communs, 132 spécifiques aux EC et 58 spécifiques aux larmes. Avec l'identification de 262 lipides répartis en 24 classes, la méthode d'extraction par IPA était la plus performante.
Conclusion : Ces résultats confirment la faisabilité de l'analyse métabo-lipidomique à partir d'EC. La réalisation d'EC successives est source de variabilité pré-analytique. Le metabolome des cellules conjonctivales est distinct et complémentaire de celui des larmes pour l'étude de la surface oculaire. Nous proposons une procédure standardisée pour l'approche métabo-lipidomique appliquée aux EC permettant la recherche de biomarqueurs en pratique clinique.

Mots clés : métabolomique ; lipidomique ; empreinte conjonctivale ; spectrométrie de masse ; chromatographie liquide

Jury :
Président du Jury : Professeur Pierre-Jean PISELLA

Directeur de thèse : ***Docteur Raoul Kanav KHANNA***

Membres du Jury : Professeur Hélène BLASCO
Docteur Guillaume VANDERMEER

Date de soutenance : 31 mai 2024