Development and application of a simple non-invasive method for *in situ* skin volatiles sampling

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The motivation and objective

Motivation:

- Compounds released from the skin provide critical information of the skin status which is useful for the disease diagnosis or biomarkers discovery.
- Current skin volatiles sampling methods have major limitations and thus fail to provide accurate information on the skin volatiles.

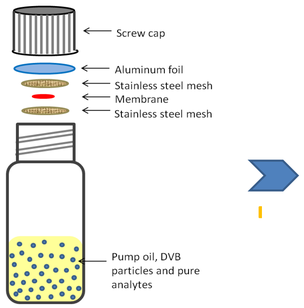
Objective:

- Develop a simple convenient and non-invasive sampling method specifically for skin volatile compounds using thin film microextraction phase.

The method development

1. *In vitro* and *in situ* sampling device set-up
2. Evaluation of the *in vitro* and *in situ* sampling device
3. Comparison of direct and headspace extraction
4. Membrane size effect
5. Monitoring skin volatiles emission from different part of the body
6. Storage method evaluation

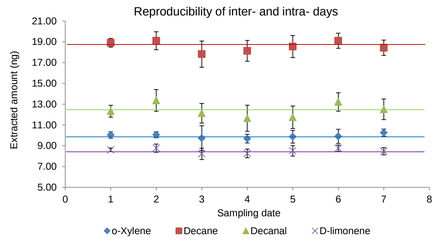
The *in vitro* device set-up and evaluation



In vitro skin mimic system

Reproducibility of the *in vitro* skin mimic system

Compounds	Inter-membrane RSD% (n=7)	Intra-day/membrane RSD% (n=6)	Inter-day RSD% (n=7)
Hexanal	4.8	2.5	3.1
Ethylbenzene	5.1	4.3	2.3
p-Xylene	4.8	3.4	*2.2*
o-Xylene	4.5	3.1	2.0
Decane	3.7	2.2	2.7
Octanal	7.4	9.8	9.6
D-limonene	3.6	2.1	2.6
Undecane	3.5	4.0	3.4
Nonanal	8.2	8.1	8.5
Dodecane	4.0	5.5	4.5
Decanal	5.0	4.6	5.4
Tridecane	4.6	6.9	5.2



- Sample matrix: 0.5 - 3 mg pure compounds into the 10 g pump oil and 4.5 g DVB mixture.
- Sample time: 5 min
- Sample temperature: 40 ºC

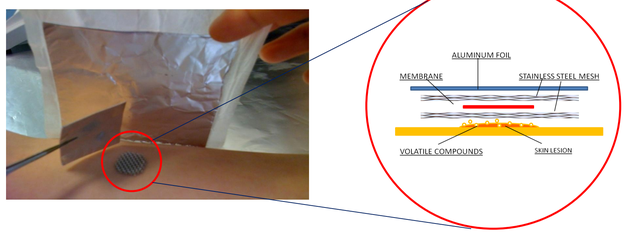
Results:
The in vitro skin mimic system was stable.
The skin sampling device was very reproducible

The *in situ* device set-up and evaluation

The reproducibility for the in vivo sampling

Chemical Name	Same sampling time * RSD% (n=4)	Different sampling time ** RSD% (n=4)
Hexanal	9	13
Nonane	2	28
Heptanal	10	24
5-Hepten-2-one, 6-methyl-	10	18
Decane	7	8
Octanal	10	10
Nonanal	13	13
Cyclooctane, methyl-	14	13
Decanal	6	6
3',4',5,7-Tetramethoxyflavone	12	11
Undecanal	1	7
Dodecanal	7	13
5,9-Undecadien-2-one, 6,10-dimethyl-	10	21
1-Dodecanol	13	12
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	14	16
Isopropyl Myristate	17	26
4,8,12-Tetradecatrienal, 5,9,13-trimethyl-	5	29
Galaxolide	22	26
Nonadecane	8	18
Hexadecanoic acid, methyl ester	20	19
Phthalic acid, butyl 2-pentyl ester	25	30
Eicosane	7	40
Isopropyl Palmitate	13	64

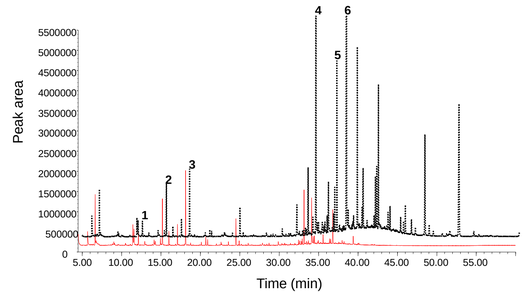
* Same sampling time: The sampling was conducted at the same time by placing four membranes on top of the skin.
** Different sampling time: Sampling performed sequentially at an hour interval



Sample area/position: Forearm
Sample time: 60 min

Results: The different time sampling shows higher RSD than the same time sampling. the reason is the influence of the environment changed and the metabolism of the skin.

Comparison of direct and headspace sampling



Comparison of the chromatograms obtained by direct (Blank dot line) and headspace (red solid line) sampling. 1, Octanal; 2, Nonanal; 3, Decanal; 4, 1-Tetradecanol; 5, Isopropyl palmitate; 6, 1-Octadecanol

Direct sampling (DS): membrane was directly placed on top of the skin surface without using the mesh.

Headspace sampling: a piece of mesh was used to separate the membrane from the contact the skin for sampling of volatiles only.

Results:

- Direct contact sampling pick up a lot of heavy compounds.
- For volatile compounds, the extraction amount of DS and HS was similar. However, DS extracts other contaminants which potentially impact on some of the volatiles.

The membrane size effect

Sample area: forearm skin
Sample time: 60 min
Membrane sizes: 5.5 mm, 11mm, 17 mm

The linear equation was obtained by plotting the membrane surface area *vs* the extraction amount from each membrane

Results:
The linearity of the membrane size *vs* the extracted amount ranges from 0.9058 to 1.000 which matches the basic principle .
In order to obtain higher sampling sensitivity, larger membrane could be used.

The linearity of the membrane size *vs* extracted amount

Compounds	Linearity Equation	R-square
5-Hepten-2-one, 6-methyl-	y = 2E+06x - 278590	0.9976
Decane	y = 68406x + 110683	0.9604
Nonanal	y = 1E+06x + 770754	1.0000
Decanal	y = 2E+06x + 962712	0.9932
Undecanal	y = 170284x + 12978	0.9712
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	y = 1E+06x + 144149	0.9973
Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	y = 2E+06x + 288271	0.9966
5,9-Undecadien-2-one, 6,10-dimethyl-	y = 4E+06x - 42816	0.9906
1-Dodecanol	y = 4E+06x - 346918	0.9886
Lilial	y = 394368x + 121190	0.9991
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	y = 1E+06x + 321910	0.9972
Diethyl Phthalate	y = 5E+06x - 2E+06	0.9351
Decane, 4-methyl-	y = 266256x - 83408	0.9474
Cyclotetradecane	y = 1E+07x - 3E+06	0.9769
n-Hexyl salicylate	y = 816526x - 136187	0.9771
Benzoic acid, 2-ethylhexyl ester	y = 310736x + 44068	0.9744
Octanal, 2-(phenylmethylene)-	y = 5E+06x - 810794	0.9802
2-Ethylhexyl salicylate	y = 3E+07x - 6E+06	0.9641
isopropyl Myristate	y = 2E+07x - 3E+06	0.9724
4,8,12-Tetradecatrienal, 5,9,13-trimethyl-	y = 3E+06x - 285504	0.9068
Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	y = 8E+06x - 872422	0.9767
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	y = 3E+06x - 773476	0.9491
4-Benzyloxybenzoic acid	y = 3E+06x - 583799	0.9796
1-Hexadecanol	y = 8E+07x - 2E+07	0.9619
Homomenthyl salicylate	y = 1E+06x - 429409	0.9805
Nonadecane	y = 3E+06x - 530702	0.9611
Hexadecanoic acid, methyl ester	y = 1E+06x - 415879	0.9640
Phthalic acid, butyl 2-pentyl ester	y = 9E+06x - 2E+06	0.9518

Conclusion

- The developed skin sampling device showed high reproducibility.
- The extraction reproducibility for *in situ* sampling may be influenced by the environmental condition and skin metabolism.
- The proposed *in situ* sampling device can be selectively used for skin volatile and semi-volatiles sampling.
- Higher sampling sensitivity can be improved using larger extraction phase depending on project objective.