Electronic Supplementary Material (ESI) for Analytical Methods.

Untargeted metabolic profiling of saliva by liquid chromatography-mass spectrometry for the identification of potential diagnostic biomarkers of asthma

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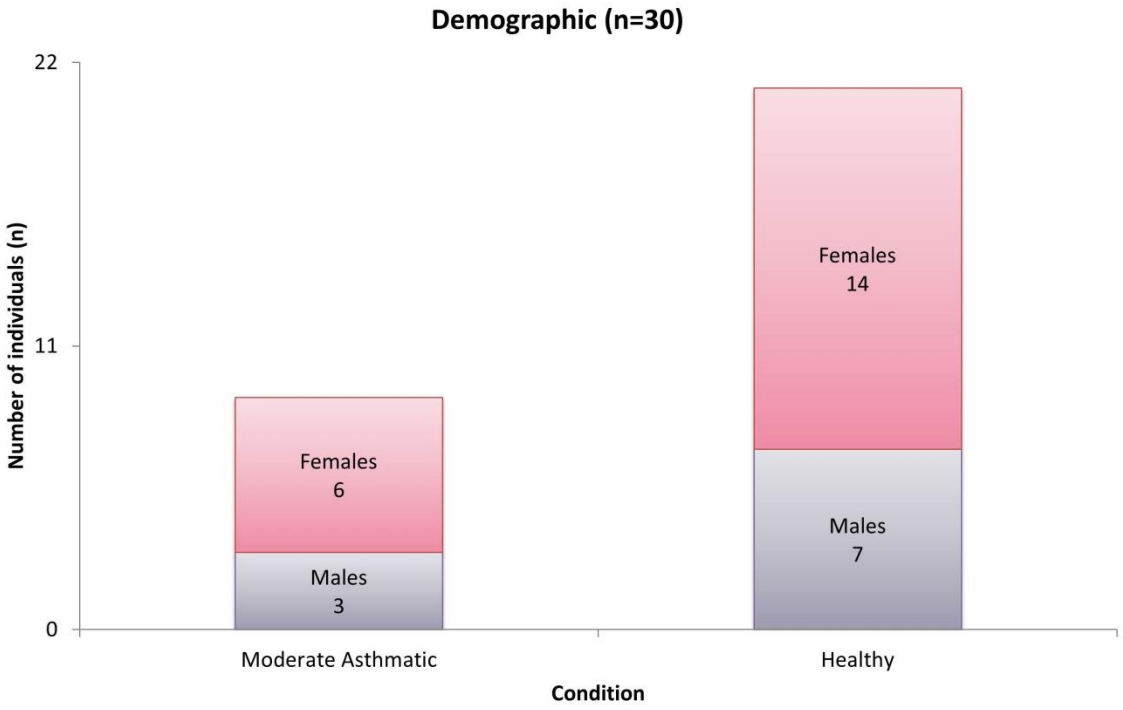
Electronic Supplementary Information



Supplementary Figure S1. Overview of demographic of the asthma metabolomic study.

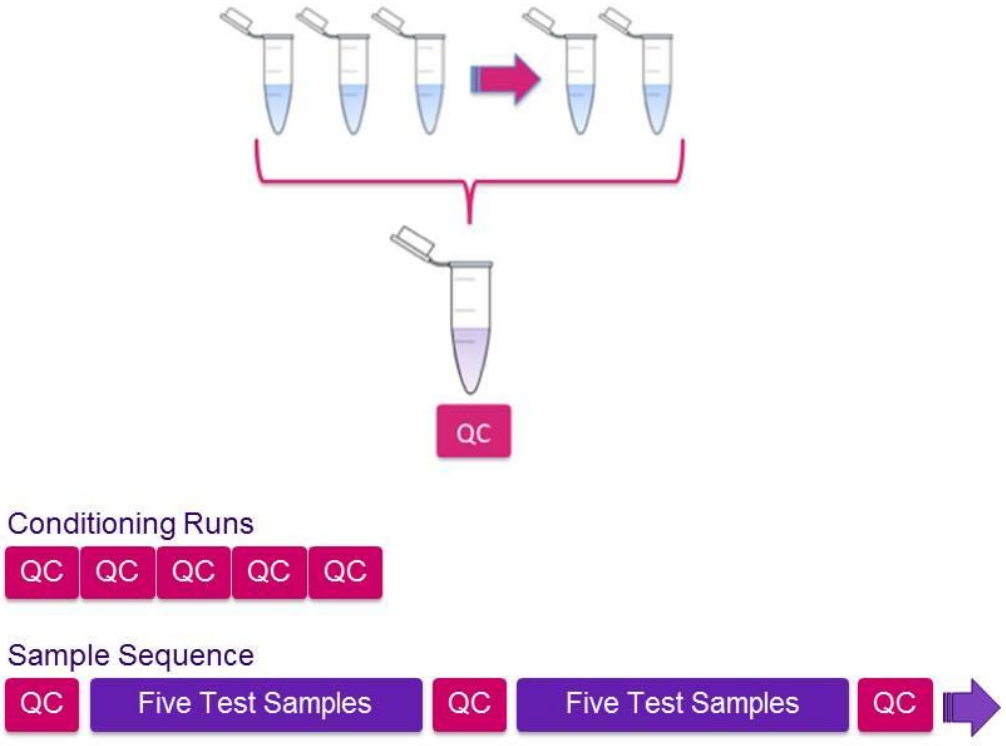
Supplementary Table S1 (a) Asthmatic patients' metadata illustrating different treatment regimens between patients, (b) Information about the inhalers used as treatments.

(a) Asthmatic patients' metadata illustrating different treatment regimes between patients

PatientID	Condition	Gender	V1FEV1% PRED	V1EOS	V1PC20	Age	Height (cm)	Weight (kg)	BDP Equiv	V1 BDP Daily	Daily Medication Routine (1)	Daily Medication Routine (2)	Total Daily ICS	BDP Equiv	Total Daily LABA
28	Asthmatic	Female	68	0.8	6.35	41	171	146.4	800	800	Symbicort 200 1BD	Oxis 6 1BD	400	400	12
10	Asthmatic	Male	67	6	2.52	60	174.5	90.5	200	200	Seretide 50 1OD	Serevent 25 1OD	50	100	25
20	Asthmatic	Female	88	1.5	2	42	149	69.2	400	400	Clenil Modulite 50 2BD	Serevent 25 2BD	200	200	100
18	Asthmatic	Male	65	4.75	0.59	52	163	63	400	400	Seretide 50 1BD	Seretide 25 1BD	100	200	50
13	Asthmatic	Female	66		0.5	28	171	81	400	400	Clenil Modulite 50 2BD	Serevent 25 2BD	200	200	100
33	Asthmatic	Male	58	19.5	2	66	173	101	2000	2000	Seretide 125 2BD		500	1000	
8	Asthmatic	Female	67		2.3	66	158	62.3	400	400	Symbicort 100/6 1BD		200	200	
5	Asthmatic	Female	59	10.75	0.03	60	162	58	1000	1000	Seretide 125 1BD	Serevent 25 1BD	250	500	50
22	Asthmatic	Female	63	0.75	1	62	173	80.6	400	400	QVAR 50 1BD	Serevent 25 1BD	100	200	50

(b) Information about the inhalers used as treatment by the asthmatic patients

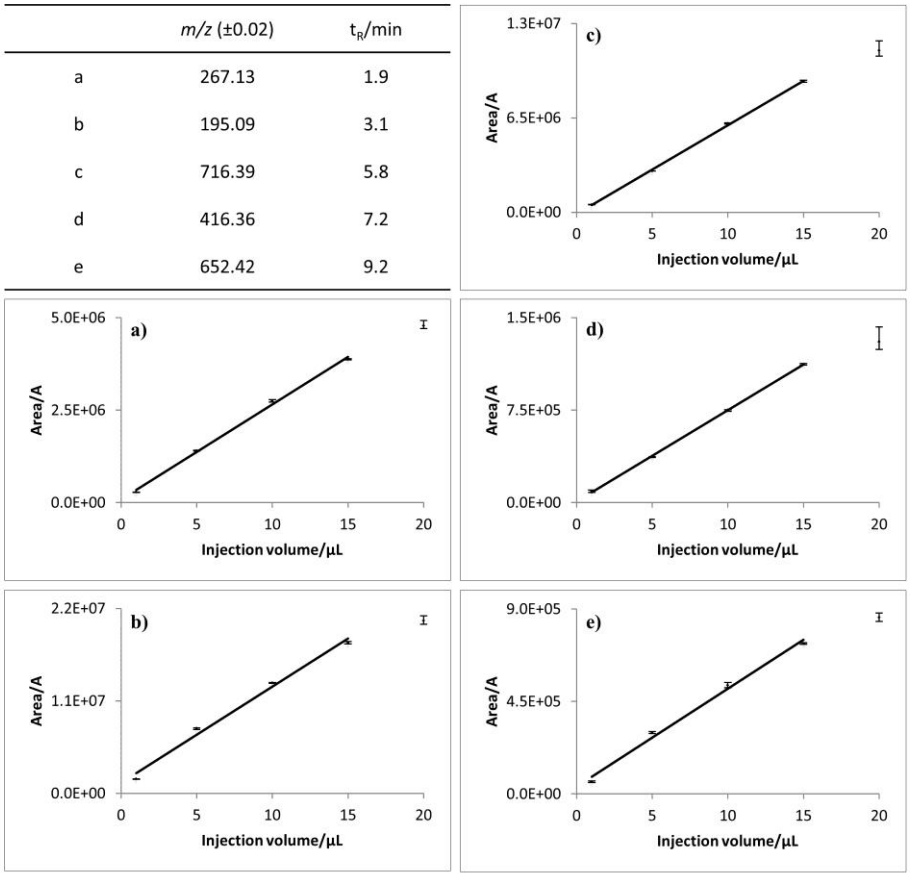
Inhaler	Device	Inhaler type	Inhaled corticosteroid	Long acting beta agonist	Propellant
Clenil Modulite	Aerosol Inhalation	MDI	Beclometasone dipropionate	NONE	HFA 134a
Seretide	Aerosol Inhalation	Evohaler	Fluticasone dipropionate	Salmeterol	HFA 134a
Serevent	Aerosol Inhalation	Evohaler	NONE	Salmeterol	HFA 134a
Symbicort	Dry Powder	Turbohaler	Budesonide	Formoterol fumarate	HFA 227
QVAR	Aerosol Inhalation	MDI	Beclometasone dipropionate	NONE	HFA 134a
Oxis	Dry Powder	Turbohaler	NONE	Formoterol fumarate	NONE
Pulmicort	Dry Powder	Turbohaler	Budesonide	NONE	NONE



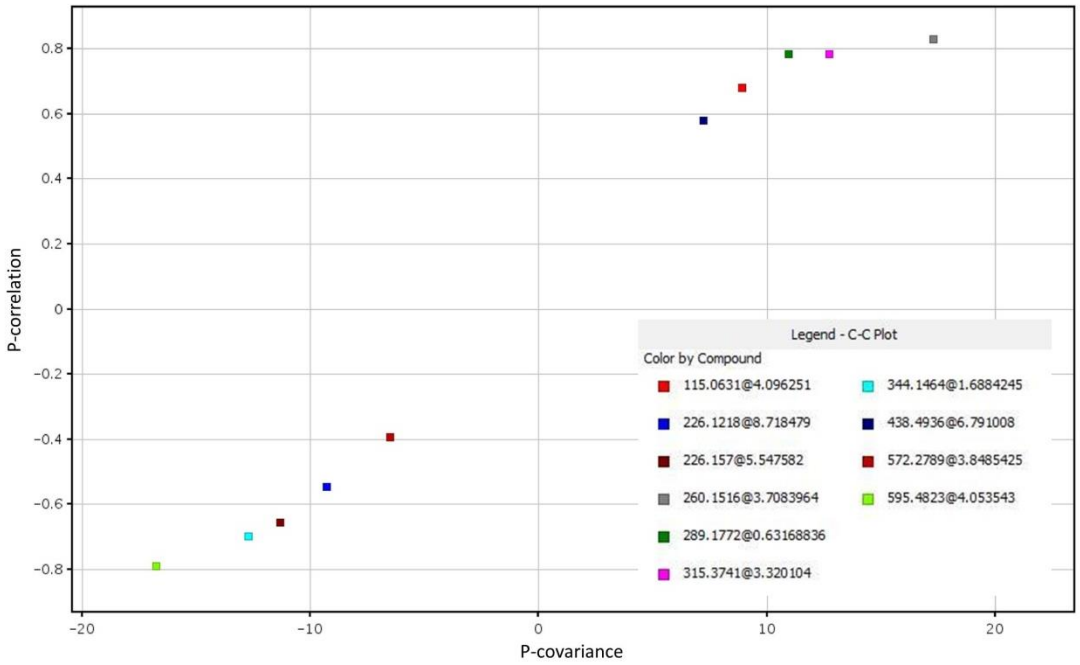
Supplementary Figure S2. Workflow used in the metabolic profiling of saliva.

Supplementary Table S2. Chromatographic retention time and peak area reproducibility for the UHPLC-MS metabolic profiling method for selected endogenous metabolites.

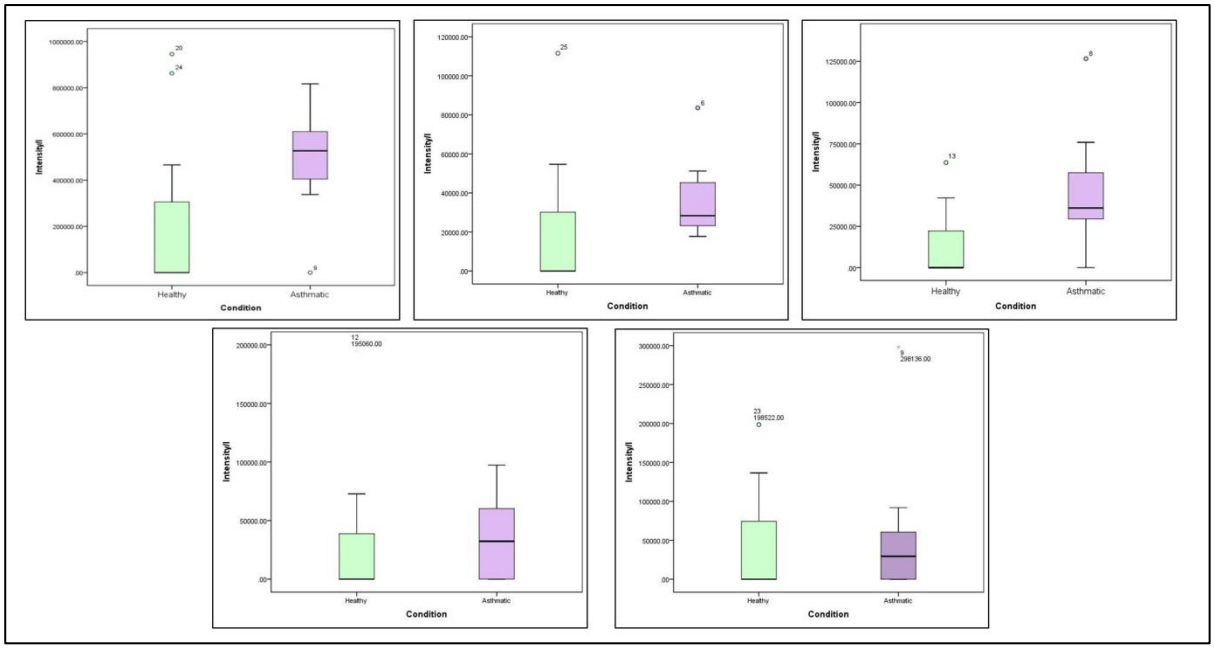
Ion *m/z* (±0.02)	**Retention Time**		**Peak area**	
	t_R (min)	**%RSD**	**Area**	**%RSD**
195.09	3.15	0.18	12909485	2.09
267.13	1.96	0.32	2005909	3.73
416.36	9.21	0.09	561820	2.76
652.42	37.04	0.08	590003	3.81
716.39	5.80	0.10	5891274	3.58



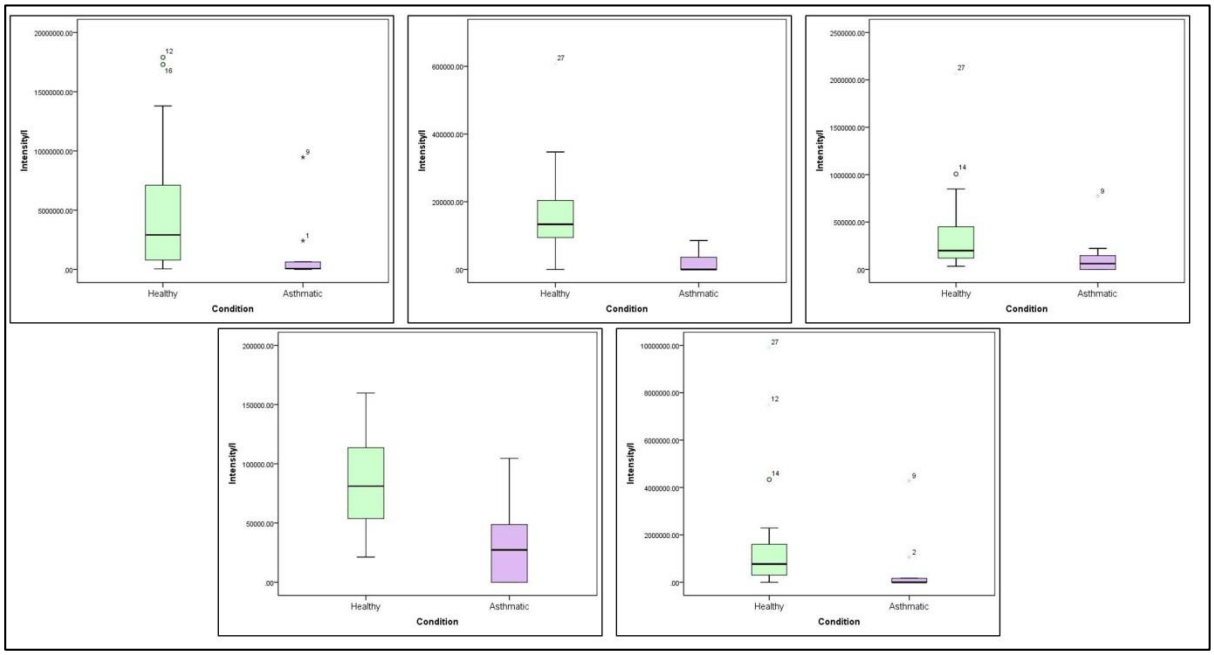
Supplementary Figure S3. Effect of injection volume for pooled saliva sample. Chromatographic peak area for selected ions across the entire chromatographic range were plotted against the injection volume. Samples were analysed in triplicates (n=3) denoted by the error bars.



Supplementary Figure S4. S-plot representing the correlation and covariance of the molecular features responsible for class separation.



Supplementary Figure S5. Box and whisker plots for Up-regulated molecular features.



Supplementary Figure S6. Box and whisker plots for Down-regulated molecular features.

Supplementary Table S3. Discriminant molecular features (MFs) obtained from moderate asthmatics versus control samples.

Retention time (min)	Measured mass	Accurate mass	Regulation	Tentative elemental composition (for ion)	Tentative annotation (HMDB entries)[a]
0.4	116.0699	116.0711	Up	$C_5H_{10}NO_2$	D/L-Proline + H^+
					Acetamidopropanal + H^+
					4-Amino-2-methylene - butanoic acid + H^+
2.2	261.1446	261.1450	Up	$C_{11}H_{20}N_2O_5$	L-Gamma-glutamyl-L-leucine + H^+
					L-Gamma-glutamyl-L-isoleucine + H^+
1.1	290.1711	290.1716	Up	$C_{12}H_{24}N_3O_5$	Metoprolol+Na^+
3.6	316.2217	316.2236	Up	$C_{15}H_{30}N_3O_4$	Not annotated
4.4	439.4569		Up	N/A[a]	Not annotated
3.4	227.1268	227.1283	Down	$C_{12}H_{19}O_4$	3,4-Methylenesebacic acid + H^+
					Allixin + H^+
4.7	573.2561	573.2603	Down	$C_{26}H_{45}N_4O_4S_3$	Not annotated
0.5	596.3253	596.3244	Down	$C_{28}H_{42}N_{11}O_2S$	Not annotated
1.7	345.1163	345.1159	Down	$C_{22}H_{18}ClN_2$	Clotrimazole + H^+
5.5	227.1396	227.1396	Down	$C_{11}H_{19}N_2O_3$	Not annotated

[a] Putative level 2 annotations[1] based on mass spectrometry physiochemical properties (5 ppm window and isotope abundance) and spectral similarity with the Human Metabolome Database spectral library.[2-4]

Citations:

1. L. W. Sumner et al., *Metabolomics* 2007, 3, 211-221.
2. D. S. Wishart, D. Tzur, C. Knox, et al., HMDB: the Human Metabolome Database. *Nucleic Acids Res.* 2007, **35**, D521-526.
3. D. S. Wishart, C. KnoxC,A. C. Guo, et al., HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res*. 2009, **37**, D603-610.
4. D. S. Wishart, T. Jewison, A. C. Guo, M. Wilson, C. Knox et al., HMDB 3.0 — The Human Metabolome Database in 2013. *Nucleic Acids Res*. 2013, **41**, D801-817.