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## Endogenous Levels of Five Fatty Acid Metabolites in Exhaled Breath Condensate to Monitor Asthma by High-Performance Liquid Chromatography: Electrospray Tandem Mass Spectrometry

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### Abstract

Airway inflammation characterizing asthma and other airway diseases may be monitored through biomarker analysis of exhaled breath condensate (EBC). In an attempt to discover novel EBC biomarkers, a high performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) method was used to analyze EBC from ten control non-asthmatics and one asthmatic individual for five fatty acid metabolites: 9,12,13-trihydroxyoctadecenoic acid (9,12,13-TriHOME), 9,10,13-TriHOME, 12,13-dihydroxyoctadecenoic acid (12,13-DiHOME), 12-hydroxyeicosatetraenoic acid (12-HETE), and 12(13)-epoxyoctadecenoic acid (12(13)-EpOME). The method was shown to be sensitive, with an on-column limit of quantitation (LOQ) in the pg range (corresponding to pM concentrations in EBC), and linear over several orders of magnitude for each analyte in the calibrated range. Analysis of EBC spiked with the five fatty acid metabolites was within 81%–119% with only a few exceptions. Endogenous levels in EBC exhibited intra- and inter-assay precision of 10%–22%, and 12%–36%, respectively. EBC from the healthy subjects contained average analyte levels between 15 and 180 pM with 12-HETE present above the LOQ in only one of the subjects at a concentration of 240 pM. Exposure of the asthmatic subject to allergen led to increased EBC concentrations of 9,12,13-TriHOME, 9,10,13-TriHOME, 12,13-DiHOME, and 12(13)-EpOME when compared to levels in

EBC collected prior to allergen exposure (range =40–510 pM). 12,13-DiHOME was significantly increased (Student's *t*-test,  $p < 0.05$ ). In conclusion, we have developed a new HPLC-ESI-MS/MS method for the analysis of five fatty acid metabolites in EBC, which are potential biomarkers for asthma monitoring and diagnosis.

## Keywords

Breath analysis; eicosanoids; exhaled breath condensate (EBC); inflammation; liquid chromatography—mass spectrometry

## I. Introduction

EXHALED BREATH CONDENSATE (EBC) has emerged as a novel matrix to assess airway inflammation [1], [2]. In contrast to bronchoalveolar lavage fluid that is collected with a fiberoptic bronchoscope [3], exhaled condensate can be collected noninvasively. The exhaled breath is simply condensed on a cold surface and collected for subsequent measurements of putative biomarkers.

Several biomarkers for airway inflammation have already been identified in EBC including eicosanoids, cytokines, and nitrogen oxide related products, all of which have the potential to provide useful information about the health status of the airways [4], [5]. Hence, asthma and other diseases characterized by chronic inflammation of the respiratory tract may some day be diagnosed and/or monitored through biomarker analysis of EBC [6]–[8]. Cysteinyl leukotrienes (LTs) were shown to increase in children with exercise-induced bronchoconstriction [9] and have also been associated with airway remodeling in childhood asthma [10]. Furthermore,  $LTB_4$  and prostaglandin (PG)  $E_2$  were increased in patients with chronic obstructive pulmonary disease [11]. In these studies, eicosanoid measurements relied on immunoassays. However, due to the low analyte levels, many commercially available immunoassay kits produce poor results [12]. Therefore, other methods such as high performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) have been developed for the simultaneous determination of eicosanoids [13] such as  $LTB_4$  [14] in EBC. Furthermore, a method for HPLC-ESI-MS analysis of 23 eicosanoids representing arachidonic acid metabolites from all three enzymatic pathways, cyclooxygenase (COX), lipoxygenase, and cytochrome P450, was recently developed and validated [15].

Linoleic acid metabolites such as the cytochrome P450 derived 12(13)-epoxyoctadecenoic acid (12(13)-EpOME), a monoepoxide also known as isoleukotoxin, and its diol 12,13-dihydroxyoctadecenoic acid (12,13-DiHOME) may be important lipid mediators regulating inflammation. The biosynthesis and toxicity of isoleukotoxin is presumed to be comparable to that of leukotoxin [16]. It is synthesized by neutrophils and has been recovered from the lungs of humans with acute respiratory distress syndrome [17], [18]. The toxicity may be due to the isoleukotoxin diol, which has been shown to disrupt mitochondrial function and cause cytotoxicity [19], [20]. Other important inflammatory mediators include the linoleic acid metabolites 9,12,13-trihydroxyoctadecenoic acid (9,12,13-TriHOME), and 9,10,13-TriHOME as well as the autooxidation product of arachidonic acid, 12-hydroxyeicosatetraenoic acid (12-HETE), which has the ability to aggregate neutrophils [21].

Due to their roles in inflammation and involvement in inflammatory lung conditions, we aimed to develop a novel methodology to quantitatively assay for 12-HETE, 12(13)-EpOME, 12,13-DiHOME, 9,12,13-TriHOME, and 9,10,13-TriHOME (see Table I). We hypothesize that this

novel set of putative biomarkers could be monitored for pulmonary diagnosis, such as for general inflammation and potentially for asthma.

## II. Experimental

### A. Chemicals

Acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ). Acetic acid, indomethacin, triphenylphosphine (TPP), ethylenediaminetetraacetic acid (EDTA), and butylated hydroxytoluene (BHT) were from Sigma-Aldrich (St. Louis, MO). Glycerol was from Difco Laboratories (Detroit, MI). Linoleic acid diol (12,13-Di-HOME) and triols (9,12,13-TriHOME, and 9,10,13-Tri-HOME) were obtained from Larodan Fine Chemicals (Malmö, Sweden), while 12-HETE, 12(13)-EpOME, and deuterated eicosanoids, *d4* - PGE<sub>2</sub> and *d8*-12-HETE were purchased from Cayman Chemical (Ann Arbor, MI). *d8*-11(12)-Epoxyeicosatrienoic acid (EET) was from Biomol International (Plymouth Meeting, PA). Deuterium exchange rate was  $\geq 99\%$  and analyte purities were  $\geq 98\%$ . 10,11-Dihydroxyheptadecanoic acid (10,11-DHHeP), and 12-[[cyclohexylamino]carbonyl]amino]-dodecanoic acid (CUDA) were synthesized in-house using standard methods [22].

### B. Exhaled Breath Condensate Collection

Ten normal healthy volunteers (five male, five female) were recruited to provide control EBC samples. Furthermore, one female who had previously been diagnosed with asthma volunteered for the study. The patient was initially diagnosed with asthma as a child in 1987 and the diagnosis was confirmed as an adult in 2004. Her prescribed therapy was a rescue inhaler and allergen avoidance, particularly down bedding. She was not on inhaled corticosteroids. EBC was collected prior to her allergen exposure and after sleeping with a down pillow and comforter for approximately 7–8 hours per night for a month (as reported by the subject).

The EBC was collected using an RTube (Respiratory Research, Charlottesville, VA). The sleeve covering the tube was kept at  $-80\text{ }^{\circ}\text{C}$  between collections. Subjects breathed into the cold tube for 10 min and 1.3–3.1 ml condensate was collected. A 1.5 ml blank was collected in an unused tube by dripping deionized water in the tube covered by the cold sleeve for 10 min.

### C. Sample Preparation

To the EBC or blank, 10  $\mu\text{l}$  antioxidant and COX-inhibitor solution was added containing 2 mg/ml indomethacin, 0.2 mg/ml EDTA, 0.2 mg/ml BHT, and 2 mg/ml TPP in methanol. The samples were stored at  $-80\text{ }^{\circ}\text{C}$ . After thawing, 8  $\mu\text{l}$  internal standard (500 nM of *d4* - PGE<sub>2</sub>, 10,11-DHHeP, *D8*-12-HETE, AND *D8*-11(12)-EET in methanol), and 6  $\mu\text{L}$  trap solution (30% (v/v) glycerol in methanol) was added. The samples were evaporated in a Speedvac (Jouan, St-Herblain, France) overnight and stored at  $-80\text{ }^{\circ}\text{C}$  until analysis. The remaining trap solution was reconstituted in 40  $\mu\text{l}$  CUDA (200 nM in methanol), vortexed, centrifuged at 14 000 r/min for 10 min, and transferred to autosampler vials.

### D. HPLC-ESI-MS/MS

An Agilent 1200 SL (Palo Alto, CA) equipped with a  $2.0 \times 150\text{ mm}$  Pursuit C18 column with a 5  $\mu\text{m}$  particle size (Varian, Lake Forest, CA) held at  $40\text{ }^{\circ}\text{C}$  was used for chromatography.

The samples were kept at  $4\text{ }^{\circ}\text{C}$  in the autosampler and the injection volume was 10  $\mu\text{l}$ . The mobile phases were (A) water with 0.1% acetic acid (v/v), and (B) a mixture of 84% acetonitrile and 16% methanol (v/v) with 0.1% acetic acid (v/v). The flow rate was 400  $\mu\text{l}/\text{min}$ . Elution of analytes was obtained with the following gradient: 15% B for 0.75 min, 15%–30% B from 0.75 to 1.5 min, 30%–47% B from 1.5 to 3.5 min, 47%–54% B from 3.5 to 5 min, 54%–55%

B from 5 to 6 min, 55%–60% B from 6 to 10.5 min, 60%–70% B from 10.5 to 15 min, 70%–80% B from 15 to 16 min, 80%–100% B from 16 to 17 min, hold 100% B from 17 to 19 min, 1.5 min re-equilibration at 15% B from 19.5 to 21 min.

An ABI QTRAP 4000 hybrid triple quadrupole/linear ion trap mass spectrometer (Foster City, CA) equipped with an electrospray ionization source was used in negative mode. The ions were scanned in the multiple reaction monitoring (MRM) mode. The instrument settings were as follows: GS1 and GS2 were 50 psi, CAD set to high, ion spray voltage was  $-4000$  V, source temperature was  $550$  °C, and declustering potential was  $-60$  V. A fragmentation prediction is found in Table I. Compound specific mass spectrometer conditions, including monitored precursor and product ion masses, are shown in Table II.

Analytes were quantified using internal standard corrected calibration curves obtained at five to seven different concentrations of a mixture containing 9,12,13-TriHOME, 9,10,13-TriHOME, 12,13-DiHOME, 12-HETE, and 12(13)-EpOME. Each analyte peak area was divided by its corresponding internal standard peak area (see Table I) and plotted against the ratio of analyte and internal standard concentration.

## E. Method Validation

*Sensitivity* for each analyte was determined by limit of quantitation (LOQ) defined as the analyte concentration corresponding to a peak response with a signal to noise ratio of at least 10. The weakest concentration of the standard mixture used to obtain the calibration curves was diluted multiple times to reach this value for each analyte. *Linearity* was demonstrated for each calibration curve determined at several occasions ( $n = 6$ ). The weighting factor  $1/x$  was applied to all calibration curves.

*Accuracy* was assessed by spiking EBC with each analyte at three different levels and comparing the results with expected values of the concentrations according to  $[C_{\text{observed}}]/[C_{\text{expected}}] \times 100$ . EBC was not concentrated in the Speedvac to avoid contribution to the expected values from endogenous analyte levels. However, matrix effects might affect the observed values and the results give the best method accuracy estimate in the absence of proper reference material for EBC.

*Method precision* (repeatability) was determined by pooling EBC from two subjects at 15 different sampling occasions. To assess intra-, and inter-assay precision, relative standard deviation (RSD) was calculated for five aliquots analyzed in a single run or 10 aliquots analyzed on two consecutive days, respectively.

*Recovery rates* of internal standards were calculated using CUDA corrected calibration curves obtained at four different concentrations of each internal standard solution. Hence, the internal standards in each sample were quantified using calibration curves normalized against the CUDA peak response and concentration in accordance with analyte quantitation described above, and the experimental values were compared to the theoretical ones. Internal standards were added to the samples in the beginning of sample preparation and CUDA right before analysis. Therefore, deviations in the internal standard experimental and theoretical values accounting for recovery rates were mainly due to internal standard losses during sample preparation under the assumption that the CUDA concentration was constant in all samples and calibrators analyzed.

### III. Results

#### A. Sensitivity and Linearity

The sensitivity of the method was characterized by LOQs in the low pg range of on-column analyte levels (see Table III). Furthermore, the linear range of each analyte was several orders of magnitude and standard errors of the slope and intercept were negligible ( $n = 6$ ).

#### B. Accuracy and Precision

Accuracy was in general between 81 and 119% for all compounds at three levels of spiked EBC (see Table IV). The highest levels of 9,12,13-TriHOME and 12,13-DiHOME were slightly outside the linear range, which accounts for a 77% accuracy. This value improved at lower concentrations, relevant to on-column levels corresponding to endogenous EBC concentrations.

Intra-assay precision expressed as relative standard deviation for three of the compounds (9,12,13-TriHOME, 9,10,13-TriHOME, and 12,13-DiHOME) was 10%–22%, and inter-assay precision was 12%–36% (see Table V). Concentrations of 12-HETE, and 12(13)-EpOME were below LOQ in the pooled sample used to assess repeatability. Method performance was further confirmed by internal standard mean recovery rates ranging between 46 and 103% displaying standard errors within 2%–8% (see Fig. 1). The trend is that the recovery decreases according to the increased non-polarity from  $d4 - PGE_2$  to  $d8-11(12)-EET$ , probably due to losses during sample preparation caused by higher affinity for plastic materials in case of more lipophilic compounds.

#### C. Levels in Exhaled Breath Condensate

Levels in EBC collected from one asthmatic subject pre-, and post-exposure to allergen, and 10 healthy subjects were measured. Average concentrations in EBC from the asthmatic subject before allergen exposure were statistically identical to the levels found in EBC from the healthy subjects according to Student's t-test (see Table VI).

Average analyte concentrations in EBC from the healthy subjects were between 15 and 180 pM. Inter-individual variability for each analyte was 22%–24% when expressed as relative standard error. 12-HETE was only present in one of the subjects at a concentration of 240 pM (see Fig. 2).

The analyte concentrations in EBC from the subject with asthma was measured periodically following allergen exposure and onset of symptoms and compared to prior exposure occasions. Representative chromatograms are shown in Fig. 3. The results revealed elevated levels of 9,12,13-TriHOME, 9,10,13-TriHOME, 12,13-DiHOME, and 12(13)-EpOME (range =40–510 pM) in the presence of asthma symptoms (see Fig. 4), with significantly elevated levels according to Student's t-test displayed by 12,13-DiHOME ( $p < 0.05$ ). In four out of six samples before allergen exposure, 12(13)-EpOME was not detected and LOQ was used for comparison to levels after allergen exposure. 12-HETE was below the LOQ at all occasions in EBC from the asthmatic subject. Intra-individual variability expressed as relative standard error was 22%–39% ( $n = 4$ ), and 4%–53% ( $n = 6$ ) with and without symptoms, respectively.

In general, sampling for 10 min corresponded to 1.3–3.1 ml EBC and the analyte levels in the EBC corresponded to an on-column concentration of 0.2–40 nM (9,12,13-TriHOME), 0.3–25 nM (9,10,13-TriHOME), 0.4–2.5 nM (12,13-DiHOME), 0.5–2.7 nM (12(13)-EpOME), and 12 nM (12-HETE). In a few occasions, the on-column concentration was below the lowest point of the calibration curve. However, the signal to noise ratio for the peak was above 10 and analyte levels were above LOQ. Then, the line equation for the calibration curve was used to

estimate the response, which facilitated quantitation. Blank levels obtained by dripping deionized water in the tube were below LOQ for all analytes, except for 9,10,13-TriHOME, partly due to instrument carry-over. However, the blank peak was negligible as compared to sample peaks.

#### IV. Discussion

The HPLC-ESI-MS/MS method we developed to analyze 9,12,13-TriHOME, 9,10,13-TriHOME, 12,13-DiHOME, 12-HETE, and 12(13)-EpOME proved to be sufficiently sensitive, accurate, and precise to determine endogenous levels in EBC from both healthy subjects and one asthmatic. However, expansion of the calibrated range for all of the analytes except 12(13)-EpOME requires further validation tests. In the current study, a few cases of quantitation above LOQ but below the lowest point in the calibration curve assumed linearity which remains to be confirmed. Hence, in future studies the standard calibration mix should be adjusted to contain lower levels of 9,12,13-TriHOME, 9,10,13-TriHOME, 12,13-DiHOME, and 12-HETE reflecting the low levels occasionally found in EBC.

Only one of the investigated analytes derived from arachidonic acid and the rest were linoleic acid metabolites. Hence, four linoleic acid metabolites that correlated with respiratory symptoms after an allergen exposure were identified (9,12,13-TriHOME, 9,10,13-TriHOME, 12,13-DiHOME, and 12(13)-EpOME). We hypothesize that this novel set of potential biomarkers may be candidates to monitor asthma. However, only one of the metabolites (12,13-DiHOME) was significantly elevated at the time of asthma symptoms emphasizing the need for a larger study with more test subjects. Furthermore, since the EBC was collected at random occasions, the intra-individual variability reflected the concentration range expected throughout a normal day with no control for eating and drinking habits. However, the samples before and after allergen exposure were collected at the same intervals throughout the day. With a more stringent sampling protocol, intra-individual variability might decrease resulting in a higher degree of significant differences between samples collected before and after allergen exposure.

The average levels of linoleic acid metabolites in EBC from healthy subjects were compared to those reported in urine from healthy individuals [23], [24]. The 9,12,13-TriHOME to 12,13-DiHOME ratio was close to ten both in urine and EBC. However, in human urine, 9,12,13-TriHOME:9,10,13-TriHOME = 10, while this ratio equaled two in EBC. Furthermore, in human urine, 12,13-DiHOME:12(13)-EpOME = 100, while these analytes were found at similar levels in EBC. Hence, different biochemical pathways may prevail in different organs (kidney and lung), reflected by different metabolite patterns in urine and EBC.

The subjects produced different volumes of EBC during the 10-min collection. However, the asthmatic subject produced similar volumes of EBC both before and after allergen exposure. The analyte concentration was presented as pM and the total amounts of exhaled fatty acid metabolites were not accounted for under the assumption that the metabolite concentration was constant throughout the collection. However, recent work has shown the collection variables may have modest effects on observed biomarker concentrations [25], and so great care was taken to standardize our EBC collection process. More research is needed to investigate possible dilution effects from the small variants of the EBC volumes. Others have emphasized the necessity for improvements in sample collection standardization and other methodological issues before use in clinical practice [5]. This has important implications for the engineering design of novel portable breath analyzers to be used in point-of-care clinical settings. Our results show that RTube in combination with LC-ESI-MS/MS are sufficient to detect fatty acid metabolites in EBC, but for these lipophilic compounds plastic or rubber collection devices might not be optimal. Potentially, the collection device containing new materials and sensor

modules for analysis could be miniaturized and interfaced. Future research may therefore be devoted to adaptation of already existing instrumentation to handheld devices.

## V. Conclusion

In conclusion, a new HPLC-ESI-MS/MS method for analysis of five fatty acid metabolites in EBC was developed with potential for miniaturization. Concentrations after allergen exposure causing asthma symptoms as well as healthy levels were investigated as a first step towards assessment of occupational health and other potential applications. While the explicit participation in reaction mechanisms typical for asthma and related airway diseases remains to be established, we have showed the potential to use HPLC-ESI-MS/MS for a novel set of EBC biomarkers useful for airway status assessment.

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**Cristina E. Davis** received the B.S. degree with a double major in mathematics and biology from Duke University, Durham, NC, in 1994, and the M.S. and Ph.D. degrees from the University of Virginia, Charlottesville, VA, in 1996 and 1999, respectively, in biomedical engineering.

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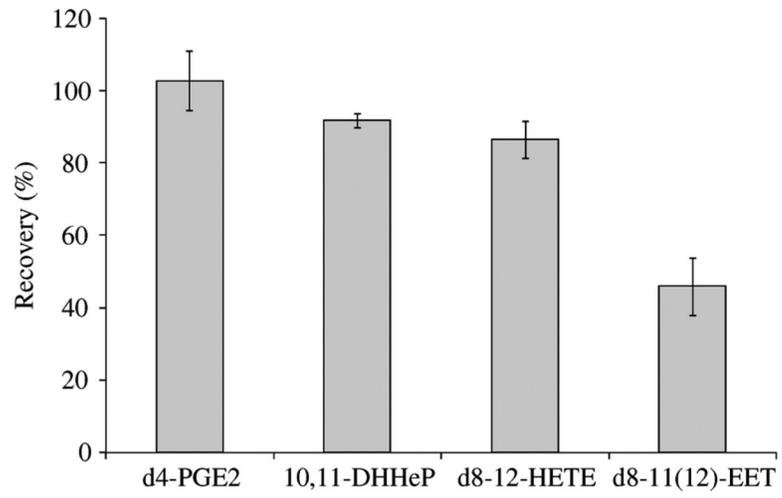


**Bruce D. Hammock** is a Distinguished Professor of Entomology with the University of California, Davis, and the Cancer Center of UCDMC. He is a member of the U.S. National Academy of Sciences. His laboratory has worked in both global metabolomics and in pathway selective metabolomics. He is particularly interested in the arachidonate cascade and regulation of pulmonary inflammation and asthma. A pharmaceutical based on this work is in clinical trials and has recently been shown to be synergistic with other compounds being developed to treat asthma.

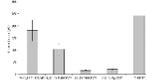
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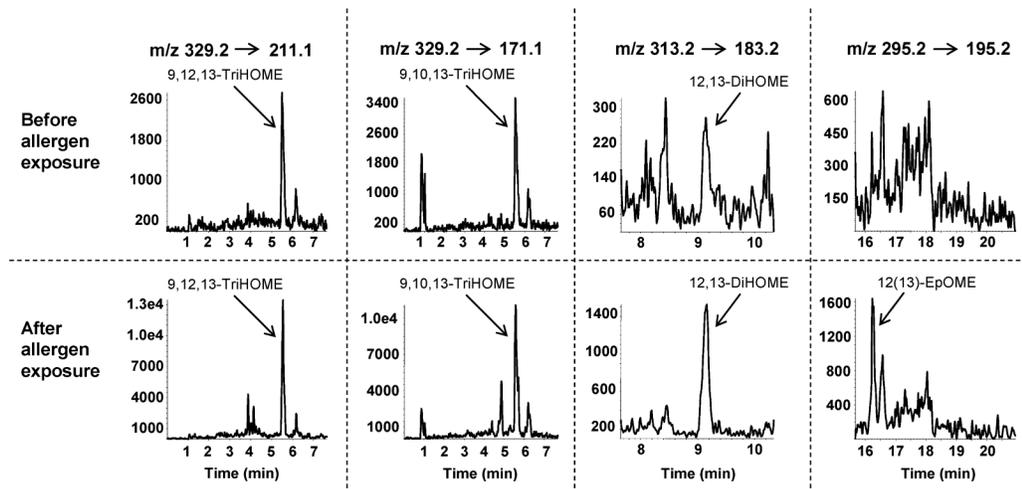
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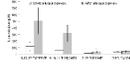
**Fig. 1.**  
Internal standard recovery rate  $\pm$  standard error ( $n = 14$ ).



**Fig. 2.** Inter-individual variability represented by average levels  $\pm$  standard error for 9,12,13-TriHOME ( $n = 10$ ), 9,10,13-TriHOME ( $n = 10$ ), 12,13-DiHOME ( $n = 10$ ), 12(13)-EpOME ( $n = 9$ ), and 12-HETE ( $n = 1$ ).



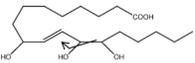
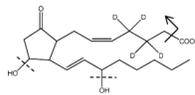
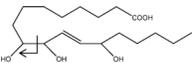
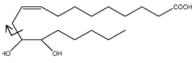
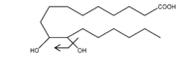
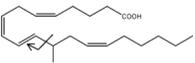
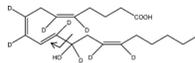
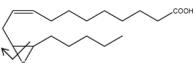
**Fig. 3.** Representative chromatograms of fatty acid metabolites found in exhaled breath condensate from an asthmatic subject before allergen exposure (upper panel) and after allergen exposure (lower panel).



**Fig. 4.** Intra-individual variability represented by average levels  $\pm$  standard error for an asthmatic subject following allergen exposure and onset of symptoms ( $n = 4$ ) compared to prior exposure occasions ( $n = 6$ ). Asterisk indicates significance in comparison to symptom free conditions ( $p < 0.05$ ).

TABLE I

Analyzed Compounds and Their Respective Internal Standard Used for Quantitation With Arrows Indicating Fragmentation

Compound	Internal Standard
 9,12,13-trihydroxyoctadecenoic acid (9,12,13-TriHOME)	 <i>d</i> <sub>4</sub> -prostaglandin (PG) E <sub>2</sub> <i>d</i> <sub>4</sub> -PGE <sub>2</sub>
 9,10,13-trihydroxyoctadecenoic acid (9,10,13-TriHOME)	
 12,13-dihydroxyoctadecenoic acid (12,13-DiHOME)	 10,11-dihydroxyheptadecanoic acid (DHHep)
 12-hydroxyicosatetraenoic acid (12-HETE)	 <i>d</i> <sub>4</sub> -12-HETE
 12(13)-epoxyoctadecenoic acid (12(13)-EpOME)	 <i>d</i> <sub>4</sub> -11(12)-epoxyicosatrienoic acid (EET)

**TABLE II**

## Compound Specific Mass Spectrometer Conditions

Compound	RT (min)	Precursor ion (amu)	Product ion (amu)	CE (eV)
9,12,13-TriHOME	5.6	329.2	211.1	-25
9,10,13-TriHOME	5.7	329.2	171.1	-25
<i>d4</i> -PGE <sub>2</sub>	5.9	355.2	275.3	-27
CUDA	8.8	339.1	214.3	-33
12,13-DiHOME	9.2	313.2	183.2	-25
DHHeP	9.3	301.2	283.3	-32
<i>d8</i> -12-HETE	14.9	327.2	184.2	-25
12-HETE	15.0	319.2	179.2	-20
12(13)-EpOME	16.4	295.2	195.2	-26
<i>d8</i> -11(12)-EET	17.0	327.2	171.2	-25

TABLE III

SENSITIVITY AND LINEARITY OF CALIBRATION CURVES ( $n = 6$ )

Compound	LOQ (pg)	LOQ (nM)	Linear range (nM)	Slope	Intercept	R
9,12,13-TriHOME	0.01	0.004	2.5 - 200	$0.351 \pm 0.004$	$0.004 \pm 0.001$	0.99
9,10,13-TriHOME	0.01	0.002	1.0 - 400	$0.671 \pm 0.019$	$0.006 \pm 0.001$	0.99
12,13-DiHOME	0.6	0.2	3.9 - 310	$0.736 \pm 0.013$	$0.007 \pm 0.002$	0.99
12-HETE	0.6	0.2	1.2 - 470	$1.530 \pm 0.018$	$0.009 \pm 0.001$	0.99
12(13)-EpOME	1.5	0.5	0.5 - 500	$19.3 \pm 0.43$	$0.03 \pm 0.01$	0.99

TABLE IV

Accuracy of Spiked Exhaled Breath Condensate

Compound	Level 1		Level 2		Level 3	
	Concentration (nM)	Recovery (%)	Concentration (nM)	Recovery (%)	Concentration (nM)	Recovery (%)
9,12,13-TrHOME	10	99	100	81	250	77
9,10,13-TrHOME	4	145	40	108	100	105
12,13-DiHOME	15	95	160	101	390	93
12-HETE	5	111	50	111	120	102
12(13)-EpOME	2	103	20	119	50	135

**TABLE V**

Precision of Endogenous Levels in Pooled Exhaled Breath Condensate

Compound	Intra-assay precision (n=5) RSD (%)	Inter-assay precision (n=10) RSD (%)
9,12,13-TriHOME	10.3	12.4
9,10,13-TriHOME	18.3	24.5
12,13-DiHOME	22.1	35.9

**TABLE VI**Average Concentration  $\pm$  Standard Error (pM) in Exhaled Breath Condensate

Compound	Subject	
	Healthy	Asthmatic pre-exposure
9,12,13-TriHOME	180 $\pm$ 43	120 $\pm$ 65
9,10,13-TriHOME	100 $\pm$ 23	66 $\pm$ 34
12,13-DiHOME	15 $\pm$ 3	17 $\pm$ 4
12(13)-EpOME	20 $\pm$ 4	35 $\pm$ 13