Figure 1

Abstract: The present invention relates to the identification of markers for the disease conditions related to asthma. The exhaled air of the patient is analysed with GC-MS and the amount of asthma markers in the breath is used to diagnose asthma. The markers used are carbon disulfide (CS2), 1-penten-2-one, butanoic acid, 3-(1-methylethyl)benzene and benzoic acid and hydrocarbons with the empirical formulas C13H28 and C11H24.
METHOD FOR THE DIAGNOSIS OF ASTHMA BY DETECTING VOLATILE ORGANIC COMPOUNDS IN EXHALED AIR.

Field of the invention

The present invention relates to the identification of markers for disease conditions related to asthma. The uses of such markers in diagnosis and a novel method for their identification are disclosed herein.

Background of the invention


Young children showing symptoms like coughing, wheezing and dyspnoea may be suffering from various diseases of the respiratory tract, and it is therefore not always easy to reach the proper diagnosis [Rosias PPR et al., Pediatr Allergy Immunol 2004;15:4-19, Chung KF. Eur Respir Rev 1998;8:999-1006, Helms PJ. Pediatr Pulmonol 2001 :S21 :49-56, Hunt J. J Allergy Clin Immunol 2002;1 10:28-34, Kharitonov SA, and Barnes PJ. Biomarkers 2002;7:1-32]. New diagnostic approaches with the potential to make an early diagnosis of asthma possible, may not only greatly decrease under-diagnosis and under-treatment in true asthmatics, but also prevent over-treatment in transient wheezers.

In recent years non-invasive techniques that may be useful for the assessment of airway inflammation have been found in the analysis of exhaled breath. For instance, the collection and analysis of non-volatile compounds in exhaled breath

Also, ethane and pentane which are detectable volatile organic compounds (VOC) in exhaled air have been related to oxidative stress as a result of inflammatory conditions [Paredi P, et al., Am J Respir Crit Care Med 2002;166:S31-S37, Olopade CO, et al., Chest 1997;111:862-865, Paredi P, et al., Am J Respir Crit Care Med 2000;161:1247-1251]. In all these approaches, single or small numbers of components are analysed and attempted to be used as diagnostic tools, so far with limited success because such individual markers suffered from a low sensitivity and/or specificity.

**Summary of the invention**

We analyzed the volatile organic compounds (VOCs) in exhaled air of patients with asthma by using a gas chromatograph - time of flight - mass spectrometer (GC-TOF-MS). We found a set of markers that may be used for the diagnosis of asthma.

We were able to discriminate between patients with asthma and normal individuals by determining whether the exhaled air contained markers selected from that set.

More in particular we found that these markers can be used in the early diagnosis of asthma. Moreover, these markers are useful in the prediction of the occurrence of asthma even when no other clinical signs are apparent yet.

The method is also useful since it provides a new non-invasive, easy, safe, cost effective and fast diagnostic tool for the diagnosis of asthma. Furthermore,
the method may be useful to distinguish between healthy wheezers and asthmatic children.

Hence, the invention relates to an in vitro method of diagnosing asthma in a patient by determining the amount of at least one volatile organic compound in a sample of exhaled air derived from a patient suspected of having asthma and comparing said amount with a normal value for said volatile organic compound, wherein a difference between the normal value and the amount of said at least one volatile organic compound is indicative of asthma, wherein said volatile organic compound is selected from the group consisting of hydrocarbon \( C_{13}H_{28} \) RT 18.89, carbon disulfide \( (CS_2) \), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon \( C_{13}H_{28} \) RT 19.00, benzoic acid, and hydrocarbon \( C_{11}H_{24} \) RT 16.93.

**Detailed description of the invention**

It was possible to assess distinctive VOC profiles, which discriminated between asthma patients and controls. Based on 20 times cross validation, a correct classification of asthma patients and controls was found using a VOC selected from table 1, either alone or in combinations as disclosed herein.

**TABLE 1**

*Identification of 10 most discriminating VOCs between asthma and controls*

<table>
<thead>
<tr>
<th>VOC No.</th>
<th>Identified as</th>
<th>Chemical formula</th>
<th>Retention Time [min.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(branched) hydrocarbon</td>
<td>( C_{13}H_{28} )</td>
<td>18.89</td>
</tr>
<tr>
<td>2</td>
<td>carbon disulfide</td>
<td>( CS_2 )</td>
<td>2.99</td>
</tr>
<tr>
<td>3</td>
<td>1-penten-2-on</td>
<td>( C_5H_8O )</td>
<td>6.29</td>
</tr>
<tr>
<td>4</td>
<td>butanoic acid</td>
<td>( C_4H_8O_2 )</td>
<td>9.26</td>
</tr>
<tr>
<td>5</td>
<td>3-(1-methylethyl)-benzene</td>
<td>( C_9H_{12} )</td>
<td>14.78</td>
</tr>
<tr>
<td>6</td>
<td>(branched) hydrocarbon</td>
<td>( C_{13}H_{28} )</td>
<td>19.00</td>
</tr>
<tr>
<td>7</td>
<td>unsaturated hydrocarbon</td>
<td>( C_{15}H_{26} )</td>
<td>22.78</td>
</tr>
<tr>
<td>8</td>
<td>benzoic acid</td>
<td>( C_7H_6O_2 )</td>
<td>16.87</td>
</tr>
<tr>
<td>9</td>
<td>p-xylene</td>
<td>( C_8H_{10} )</td>
<td>11.66</td>
</tr>
<tr>
<td>10</td>
<td>(branched) hydrocarbon</td>
<td>( C_{11}H_{24} )</td>
<td>16.93</td>
</tr>
</tbody>
</table>
The compounds in table 1 can be distinguished on the basis of their chemical formula only, or in a number of cases by the combination of their chemical formula and the retention time of the compound on the specific column used (see examples section). Under the circumstances used, the retention time of toluene was 9.15 minutes. The skilled person will appreciate that the retention times as referred herein are approximate values subject to the usual experimental variance.

As an example of the terminology used herein, compound number 1 is referred to as a (branched) hydrocarbon $\text{C}_{13}\text{H}_{28}$RT 18.89. This means a hydrocarbon which may or may not be branched with a retention time on gas-chromatography with a Restek RTX-5ms column, 30m x 0.25 mm ID, coated with 1.0 um HP-5 phase when helium was used as the carrier gas at a flow rate of 1.5 ml/min.

It was found that any of the compounds in table 2 on its own was capable of discriminating between healthy persons and asthma patients. This is also illustrated in figure 1.

<table>
<thead>
<tr>
<th>VOC No</th>
<th>Identified as</th>
<th>Chemical formula</th>
<th>Retention Time [min.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(branched) hydrocarbon</td>
<td>$\text{C}<em>{13}\text{H}</em>{28}$</td>
<td>18.89</td>
</tr>
<tr>
<td>2</td>
<td>carbon disulfide</td>
<td>$\text{CS}_{2}$</td>
<td>2.99</td>
</tr>
<tr>
<td>3</td>
<td>1-penten-2-on</td>
<td>$\text{C}_5\text{H}_8\text{O}$</td>
<td>6.29</td>
</tr>
<tr>
<td>4</td>
<td>butanoic acid</td>
<td>$\text{C}_4\text{H}_8\text{O}_2$</td>
<td>9.26</td>
</tr>
<tr>
<td>5</td>
<td>3-(1-methylethyl)-benzene</td>
<td>$\text{C}<em>9\text{H}</em>{12}$</td>
<td>14.78</td>
</tr>
<tr>
<td>6</td>
<td>(branched) hydrocarbon</td>
<td>$\text{C}<em>{13}\text{H}</em>{28}$</td>
<td>19.00</td>
</tr>
<tr>
<td>8</td>
<td>benzoic acid</td>
<td>$\text{C}_7\text{H}_6\text{O}_2$</td>
<td>16.87</td>
</tr>
<tr>
<td>10</td>
<td>(branched) hydrocarbon</td>
<td>$\text{C}<em>{11}\text{H}</em>{24}$</td>
<td>16.93</td>
</tr>
</tbody>
</table>

Hence, the invention relates to an in vitro method of diagnosing asthma in a patient by determining the amount of at least one volatile organic compound in a sample of exhaled air derived from a patient suspected of having asthma and comparing said amount with a normal value for said volatile organic compound, wherein a difference between the normal value and the amount of said at least one volatile organic compound is indicative of asthma, wherein said volatile
organic compound is selected from the group consisting of hydrocarbon \(\text{C}_3\text{H}_2\text{S}\) RT 18.89, carbon disulfide (CS\(_2\)), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon \(\text{C}_3\text{H}_2\text{S}\) RT 19.00, benzoic acid and hydrocarbon \(\text{CnH}_{24}\) RT 16.93.

The volatile organic compounds thus characterized by their molecular formula may be directly detected by other detection means as the ones mentioned herein. Alternative methods include an electronic nose, surface Plasmon resonance or other means. The volatile organic compounds characterized by their molecular formula in combination with their retention times may also be identified by other means than the ones identified herein. For that purpose, the compounds in an individual peak as detected by the methods described herein may be isolated and used to calibrate the alternative method of choice.

Said sample derived from a patient is a sample of exhaled air directly obtained from the patient, it may also be a sample wherein the volatile organic compounds in the exhaled air are concentrated, collected and/or immobilized, such as an active carbon solid phase or an affinity matrix.

A volatile organic compound as defined herein is an organic compound which can be detected in the method as described herein, i.e. an organic compound is volatile if it is capable of being detected in the gas phase, for instance when a gas chromatograph may be used for its detection.

A method according to the invention makes a contribution to the art as a whole, in that it provides a method with a sensitivity of more than 45% while the specificity is more than 85%. The prior art methods do not provide such high specificity and/or sensitivity.

Methods to detect VOCs in exhaled air as such are known in the art. The examples provide guidance for the skilled person to perform an analysis according to the invention. Normal values may be obtained by determining VOCs in the exhaled air of normal individuals. VOCs may be detected in several ways known in the art. This may be done by a device capable of measuring more than one VOC at the same time such as a mass spectrometer or by a device specifically suitable for measuring only one VOC at a time.

Figure 1 shows the mean relative intensity of the ten VOCs from table 1. It can be seen that VOCs 1, 2, 4, 5, 6, 7, and 8 increased in the population of asthma patients, whereas VOC numbers 3, 9 and 10 are decreased in the population of asthma patients when compared to normal controls. Numbers 7 and 9 when used individually did not provide significant differences between normal individuals and asthma patients when tested alone, however, in combination with each other or with at
least one of VOCs No: 1 - 6, 8 or 10, VOCs 7 and 9 contributed significantly to the
discriminative power of the method.

When a single VOC is to be used, any VOC selected from table 2
may result in a satisfactory discrimination between normal individuals and asthma
patients. However, VOC No 1, i.e. a hydrocarbon with molecular formula of C10H2S
retention time of 18.89 min is preferred. This may be advantageous in an assay
capable of detecting only a single VOC at a time such as an electronic nose. The
preferred hydrocarbon may be linear or branched.

The sensitivity, specificity, and the percentage of correct classification
increases with increasing number of VOCs selected from table 1. Hence, the invention
also relates to a method as described above wherein at least two volatile organic
compounds are detected, such as three, four, five or six VOCs. The method may even
be further improved by determining the relative amounts of seven, eight, nine VOCs.
Most preferred is a method as described above wherein all ten VOCs from table 1 are
determined in a method according to the invention.

Figure 2 shows the percentage of correct classification of asthma
with increased numbers of VOCs. It can be seen that the percentage of correct
classification increases when more than one VOC selected from table 1 is used in a
method according to the invention. The highest sensitivity and specificity could be
obtained when a method according to the invention was performed with all 10 VOCs
from table 1 combined.

A particularly advantageous method employs any of the VOCs
selected from the group consisting of VOCs #2, 3, 4, 5 and 8. These compounds are
defined by their chemical formula alone. VOCs #1, 6, 7, 9 and 10 are unequivocally
defined by their chemical formula in combination with their retention times on the
particular GC column as used herein.

Advantageously, when 2 VOCs selected from the group consisting of
VOC #2, 3, 4, 5 and 8 were used in a method according to the invention, the sensitivity
and/or specificity of the method improved even further, this was also the case when 3,
4 or 5 VOCs selected from that group were used.

A preferred embodiment of the method according to the invention
comprises the use of VOC No 1 together with a VOC selected from the group
consisting VOC No 2 to VOC No. 10, preferably No 2.

When the discriminant analyses was performed on 95% of the
chromatograms (training set), leaving the remaining 5% (test set) as unknowns to be
classified using the training model, 6 VOCs were sufficient to reach a sensitivity and a
specificity of both 84%, 8 VOCs were sufficient to reach 89% sensitivity at 95% specificity whereas 100% sensitivity could be reached when all VOCs from table 1 were used (figure 2).

VOC can easily be determined by collection of exhaled breath and quantitative analysis by thermal desorption-gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) [Van Berkel JJBN, et al., J Chrom B 2008; 861:101-7].

A method according to the invention may be particularly advantageous for the diagnosis of asthma in children below 6 years of age, being an age group in which a definitive diagnosis based on doctors' examination is still difficult. Since under-diagnosis and under-treatment in true asthmatics and over-treatment in transient wheezers are undesirable from a medical standpoint and in terms of public health, an earlier diagnosis would be valuable.

A method according to the invention may also be used to distinguish atopic asthma patients from healthy persons. The results of the analyses in which breath samples of atopic and non-atopic asthma patients were compared, indicate that not only healthy from diseased can be distinguished in this way, but that it is also possible to differentiate one form of disease from another.

Legend to the figures

Figure 1 shows the mean relative intensities of the ten VOCs listed in table 1 which were capable to distinguish between asthmatic and healthy individuals. Figure 2 shows the percentage of correct classification of asthma with increasing numbers of VOCs.

Examples

Example 1: Study population

A total of 120 children in the age range of 5 - 16 years were included in the study. Of these children 63 were diagnosed with asthma and 57 were healthy controls. The asthmatic children were recruited from the outpatient clinic of the department of paediatric pulmonology of the University Hospital Maastricht. The asthma diagnosis was made on clinical grounds by an experienced children's lung specialist.

Asthma was defined as a chronic inflammatory disorder with intermittent symptoms of cough, dyspnoea, wheezing and chest pain. Severity and control of asthma were assessed according to the international Global Initiative for Asthma (GINA) guidelines [Global Initiative for asthma (GINA). Pocket guide for

A subset of 42 atopic asthma patients was selected, based on an established positive Phadiatop and/or at least two RAST classes equal to or greater than 2. Appropriate therapy was prescribed by the patient's own physician. All 63 patients used an inhaled bronchodilator on demand and 55 of them used an inhaled corticosteroid daily. Sixty seven percent of this population had allergy as documented by radio allergosorbent test (RAST) class 2 or higher for at least one common airborne allergen.

The 57 control subjects were recruited from primary schools (36 out of 57) and from the outpatient paediatric clinic of the University Hospital Maastricht (21 out of 57). In this group enuresis and constipation were the main reasons for consultation.

The ISAAC questionnaire was used to exclude a history of respiratory problems in these children [Variations in the prevalence of respiratory symptoms, self-reported asthma attacks and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). Eur Respir J 1996; 9:687-95]. Patients with diseases that might interfere with the results of this study, mentally retarded children and active smokers were excluded from the study. The two populations were comparable in age, height and weight.

Characteristics of patients and controls are presented in table 3. Informed consent was obtained from all children and their legal representatives. The study was approved by the Ethics Committee of Maastricht University, clinical trial registration number NCT 00413140.

Table 3 Characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Asthma (n=63)</th>
<th>Controls (n=57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>10.8 ± 3.1</td>
<td>9.9 ± 2.8</td>
</tr>
<tr>
<td>Height</td>
<td>144 ± 17</td>
<td>141 ± 17</td>
</tr>
<tr>
<td>Weight</td>
<td>38.2 ± 14.9</td>
<td>34.8 ± 10.9</td>
</tr>
<tr>
<td>Gender male/female</td>
<td>47 / 16</td>
<td>29 / 28</td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>96.2 ± 18.2</td>
<td>101.8 ± 10.6</td>
</tr>
<tr>
<td>FEV1 / VC (%)</td>
<td>84.0 ± 10.3</td>
<td>88.2 ± 7.0</td>
</tr>
<tr>
<td>Atopy</td>
<td>42 (67%)</td>
<td></td>
</tr>
<tr>
<td>Asthma severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermittent</td>
<td>15 (24%)</td>
<td></td>
</tr>
</tbody>
</table>
Severity was classified according to Global Initiative for Asthma (GINA)

Significantly different (p=0.01). No confounding of gender on the discriminant functions for asthma vs. control and atopic vs. non-atopic asthma could be established.

Example 2: Sampling

The subjects were asked to exhale their breath in a resistance free plastic bag (Tedlar bag, SKC ltd., Dorset, UK). Three to four exhalations were sufficient to fill the 5-litre bag. Neither special provisions for the manner of exhalation, nor special directions concerning the diet of the subjects were given. The samples of both, patients and controls were collected in the same room.

Within one hour of collection, the bag was emptied under pressure over a stainless steel two-bed sorption tube, filled with carbograph 1TD/Carbopack X (Markes International, Llantrisant, Wales, UK). Before and after loading the tubes were air-tight capped. The tubes were stored at room temperature until analysis.

All patients were instructed to withhold from using their inhaler during 8 hours prior to the sampling.

Example 3: VOC Analysis

Analysis of the samples was performed as previously described [Van Berkel JJBN, et al., J Chrom B 2008;861:101-7]. In brief; the volatile compounds trapped on the sorption tubes by thermal desorption were released, using the Markes International Ultra-Unity automated thermal desorption equipment (Markes International, Llantrisant, Wales, UK). The gaseous mixture of released compounds was then split; 90% of the sample was recollected on a second identical sample tube and stored for an optional second analysis. 10% of the sample was loaded onto a cold (5 °C) sorption trap, from which it was injected into a gas chromatograph (Trace GC, Thermo Fischer Scientific, Austin, Texas, USA) and analysed by time-of-flight mass spectrometry (Tempus Plus, Thermo Fischer Scientific, Austin, Texas, USA).

For the GC-MS measurements the following conditions were used:

<table>
<thead>
<tr>
<th>Severity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>mild persistent</td>
<td>12 (19%)</td>
</tr>
<tr>
<td>moderate persistent</td>
<td>17 (27%)</td>
</tr>
<tr>
<td>severe persistent</td>
<td>19 (30%)</td>
</tr>
<tr>
<td>allergic rhinitis</td>
<td>11 (17%)</td>
</tr>
<tr>
<td>ICS use</td>
<td>55 (87%)</td>
</tr>
<tr>
<td>ICS daily dose (μg)</td>
<td>576 ± 357</td>
</tr>
</tbody>
</table>

(1) Severity was classified according to Global Initiative for Asthma (GINA)
(2) Significantly different (p=0.01). No confounding of gender on the discriminant functions for asthma vs. control and atopic vs. non-atopic asthma could be established.
column: Restek RTX-5ms, 30m x 0.25 mm ID, coated with 1.0 um HP-5 phase; helium was used as the carrier gas at a flow rate of 1.5 ml/min. The temperature of the gas chromatograph was programmed as follows: 40 °C for 5 min., then increased by 10 °C/min until 270 °C, at which temperature it was maintained for 5 min. The mass spectrometer was set at a scan range of 35-350 AMU and scanned 5 times per second. The complete analytical procedure, including sampling, storage and instrumental analysis was tested for reproducibility. Instrumental reproducibility was determined by the analysis of identical exhaled air samples that were obtained by emptying a filled bag over y-shaped connector onto two absorption tubes. The two absorption tubes were subsequently analyzed by GC-TOF-MS. This experiment was repeated 6 times.

The quantification of the similarity was done by means of calculation of a distance measure (dot product rule). This distance measure is based on the similarity of the entire raw chromatogram. Distance measure calculation of all complementary files resulted in a distance measure ranging from 0.96 to 0.99. (A value of '1' denotes identical samples, the lower the value the lesser the degree of similarity). Intra-individual variability was examined by repeated sampling of exhaled air from 10 subjects for 5 consecutive days and comparing the results per subject from day to day. Inter-individual variability was examined by sampling 10 subjects and comparing the data from subject to subject. Again the similarity between several chromatograms was quantified using a distance measure as mentioned above. The intra-individual variability ranged from 0.80 to 0.99 and is far smaller than the inter-individual variability, ranging from 0.16 to 0.98

Example 4: Data analysis

The GC-MS chromatograms of the breath samples of the 120 subjects involved in this study were recorded and retention times were normalized by calculating retention indices, relative to toluene and lining up using easily recognizable component peaks, to correct for chromatographic drifting. Parts of the chromatograms that occurred at a retention index < 0.15 and at a retention index > 2.8 were removed from the chromatograms, because of unreliable data from these parts, due to noisy mass spectra at the beginning of the chromatograms and column bleeding at the end of each run.

The remaining data were transformed to Excel files, containing almost 6000 different chromatographic peaks, determined by retention time and mass spectrum and combined with a relative intensity. In our approach the measured mass
spectra were compared to one another at the same retention time, rather than to library spectra.

In this way the resemblance of the original spectra determined the decision whether peaks at the same retention time represent the same component or not. Intensities below the detection limit were set at 0%. The detailed descriptions of the data handling procedures can be found elsewhere [Van Berkel JJBN, et al., J Chrom B 2008;861 :101-7].

Before data analysis was executed, all peaks that occurred in less than 8% of the samples (meaning less than ten times in this study) were removed. This resulted in a final data matrix, containing 945 peaks and 120 subjects. This matrix was normalized by adjusting the sum of all peak intensities in each subject at 100%. This way the influence of the infrequently appearing peaks could be diminished. The resulting data were analysed by a stepwise discriminant analysis, using SPSS (SPSS13.0 for windows, SPSS Inc. Chicago, Illinois, USA).

The discriminant analyses were performed using a twentyfold cross-over approach. In this method all but 5 percent of the chromatograms (in this study 5 percent equals 6 samples) are used to construct the discriminant function, which is subsequently used to predict to which group the ones left out belong. This is repeated 20 times, until all samples have once been classified.
CLAIMS

1. An in vitro method of diagnosing asthma in a patient by determining the amount of at least one volatile organic compound in a sample of exhaled air derived from a patient suspected of having asthma and comparing said amount with a normal value for said volatile organic compound, wherein a difference between the normal value and the amount of said at least one volatile organic compound is indicative of asthma, wherein said volatile organic compound is selected from the group consisting of hydrocarbon \( \text{C}_3\text{H}_8 \) RT 18.89, carbon disulfide (\( \text{CS}_2 \)), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon \( \text{C}_5\text{H}_{26} \) RT 19.00, benzoic acid and hydrocarbon \( \text{CnH}_{24} \). RT 16.93.

2. Method according to claim 1 wherein said method comprises the step of determining at least the amount of a hydrocarbon \( \text{C}_3\text{H}_{28} \) RT 18.89 in said sample.

3. Method according to claim 2 wherein said method consists of the step of determining at least the amount of a hydrocarbon \( \text{C}_3\text{H}_{28} \) RT 18.89 in said sample.

4. Method according to claims 1 or 2 wherein at least 2 volatile organic compounds are detected, selected from the group consisting of volatile organic compounds hydrocarbon \( \text{C}_3\text{H}_{28} \) RT 18.89, carbon disulfide (\( \text{CS}_2 \)), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon \( \text{C}_5\text{H}_{26} \) RT 19.00, unsaturated hydrocarbon \( \text{C}_5\text{H}_{26} \) RT 22.78, \( \text{p-xylene} \), benzoic acid and hydrocarbon \( \text{CnH}_{24} \). RT 16.93.

5. Method according to claim 4 wherein at least 3 volatile organic compounds are detected selected from the group consisting of volatile organic compounds hydrocarbon \( \text{C}_3\text{H}_{28} \) RT 18.89, carbon disulfide (\( \text{CS}_2 \)), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon \( \text{C}_5\text{H}_{26} \) RT 19.00, unsaturated hydrocarbon \( \text{C}_5\text{H}_{26} \) RT 22.78, \( \text{p-xylene} \), benzoic acid and hydrocarbon \( \text{CnH}_{24} \). RT 16.93.

6. Method according to claim 5 wherein at least 4 volatile organic compounds are detected selected from the group consisting of volatile organic compounds hydrocarbon \( \text{C}_3\text{H}_{28} \) RT 18.89, carbon disulfide (\( \text{CS}_2 \)), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon \( \text{C}_5\text{H}_{26} \) RT 19.00, unsaturated hydrocarbon \( \text{C}_5\text{H}_{26} \) RT 22.78, \( \text{p-xylene} \), benzoic acid and hydrocarbon \( \text{CnH}_{24} \). RT
7. Method according to claim 6 wherein at least 5 volatile organic compounds are detected selected from the group consisting of volatile organic compounds: hydrocarbon C_{i}H_{9}S RT 18.89, carbon disulfide (CS₂), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon C_{i}H_{26} RT 19.00, unsaturated hydrocarbon C_{i}H_{26} RT 22.78, p-xylene, benzoic acid and hydrocarbon C_{n}H_{24}. RT 16.93.

8. Method according to claim 7 wherein at least 6 volatile organic compounds are detected selected from the group consisting of volatile organic compounds: hydrocarbon C_{i}H_{28} RT 18.89, carbon disulfide (CS₂), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon C_{i}H_{28} RT 19.00, unsaturated hydrocarbon C_{i}H_{26} RT 22.78, p-xylene, benzoic acid and hydrocarbon C_{n}H_{24}. RT 16.93.

9. Method according to claim 8 wherein at least 7 volatile organic compounds are detected selected from the group consisting of volatile organic compounds: hydrocarbon C_{i}H_{28} RT 18.89, carbon disulfide (CS₂), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon C_{i}H_{28} RT 19.00, unsaturated hydrocarbon C_{i}H_{26} RT 22.78, p-xylene, benzoic acid and hydrocarbon C_{n}H_{24}. RT 16.93.

10. Method according to claim 9 wherein at least 8 volatile organic compounds are detected selected from the group consisting of volatile organic compounds: hydrocarbon C_{i}H_{28} RT 18.89, carbon disulfide (CS₂), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon C_{i}H_{28} RT 19.00, unsaturated hydrocarbon C_{i}H_{26} RT 22.78, p-xylene, benzoic acid and hydrocarbon C_{n}H_{24}. RT 16.93.

11. Method according to claim 10 wherein at least 9 volatile organic compounds are detected selected from the group consisting of volatile organic compounds: hydrocarbon C_{i}H_{28} RT 18.89, carbon disulfide (CS₂), 1-penten-2-on, butanoic acid, 3-(1-methylethyl)-benzene, hydrocarbon C_{i}H_{28} RT 19.00, unsaturated hydrocarbon C_{i}H_{26} RT 22.78, p-xylene, benzoic acid and hydrocarbon C_{n}H_{24}. RT 16.93.
12. Method according to claim 11 wherein at least 10 volatile organic compounds are detected selected from the group consisting of volatile organic compounds: hydrocarbon \( \text{C}_3\text{H}_2\text{S} \) RT 18.89, carbon disulfide (\( \text{CS}_2 \)), 1-penten-2-on, butanoic acid, 3-(1-methylene)-benzene, hydrocarbon \( \text{C}_3\text{H}_{28} \) RT 19.00, unsaturated hydrocarbon \( \text{C}_5\text{H}_{26} \) RT 22.78, p-xylene, benzoic acid and hydrocarbon \( \text{CnH}_{24} \). RT 16.93.
Figure 2

- Sensitivity
- Specificity

Number of VOCs tested vs. sensitivity and specificity (in %)
**INTERNATIONAL SEARCH REPORT**

**International application No**  
PCT/EP2010/059670

---

### A. CLASSIFICATION OF SUBJECT MATTER

**INV.**  G01N33/497  A61B5/08

---

### B. FIELDS SEARCHED

**Minimum documentation searched** (classification system followed by classification symbols)

GOIN  A61B

---

**Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched**

---

**EPO-Internal**

---

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with Indication where appropriate, of the relevant passages</th>
<th>Relevant to claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DCWELING, E ET AL.: &quot;Profielen van vluchtige stoffen in uitademingslucht: een nieuw middel voor de diagnostiek en monitoring van chronische longziekten?&quot; NEDERLANDS TIDJSCHRIFT VOOR ALLERGIE, vol. 8, no. 5, 2008, pages 156-162, XP002549444 the whole document</td>
<td>1</td>
</tr>
</tbody>
</table>

---

**Further documents are listed in the continuation of Box C**

---

**See patent family annex**

---

**Date of the actual completion of the international search**  
22 September 2010

---

**Date of mailing of the international search report**  
29/09/2010

---

**Name and mailing address of the ISA/ European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV ROSWIJK Tel (+31-70) 340-2040, Fax (+31-70) 340-3016**  
Steinmetz, Johannes
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DRAGONIERI ET AL: &quot;An electronic nose in the discrimination of patients with asthma and controls&quot; JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, MOSBY - YEARLY BOOK, INC, US, vol. 120, no. 4, 1 October 2007 (2007-10-01), pages 856-862, XP022287291 ISSN: 0091-6749 the whole document</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>PAREDI, P; KHRITONOV, SA; BARNES, PJ: &quot;Elevation of exhaled ethane concentration in asthma&quot; AM J RESPIR CRIT CARE MED, vol. 162, 2000, pages 1450-1454, XP002549445 * abstract</td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>OLOPADE, CO; ZAKKAR, M; SWEDLER, WI; RUBINSTEIN, I: &quot;Exhaled pentane levels in acute asthma&quot; CHEST, vol. 111, no. 4, April 1997 (1997-04), pages 862-865, XP002549446 * abstract</td>
<td>1</td>
</tr>
<tr>
<td>Category</td>
<td>Citation of document, with indication, where appropriate, of the relevant passages</td>
<td>Relevant to claim No</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>US 7101340 B1</td>
<td>05-09-2006</td>
<td>NONE</td>
</tr>
</tbody>
</table>