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PROTOZOOLOGY - ORIGINAL PAPER

Identification of volatile biomarkers of *Giardia duodenalis* infection in children with persistent diarrhoea

C. Ubeda[1] · E. Lepe-Balsalobre[2] · C. Ariza-Astolfi[2] · J. M. Ubeda-Ontiveros[2]

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Abstract

Currently, chronic diarrhoea syndrome in children is a very common pathology whose aetiology is sometimes difficult to identify. Methodologies for the diagnosis of infections have diversified, and gas chromatography/mass spectrometry (GC/MS) is a very useful tool. The aim of this study was to identify volatile biomarkers of the presence of *Giardia duodenalis* in the faeces of patients with chronic diarrhoea (with and without giardiasis) using static headspace extraction followed by GC/MS. The analysis of the volatiles extracted from the headspace had enough sensitivity to detect differences in the volatile profiles in the faeces of the patients with and without *Giardia duodenalis* infection and discriminate between them. Dimethyl disulphide and trisulphide were found in the faeces of patients without giardiasis but not in the faeces of patients with *G. duodenalis.* Finally, three possible biomarkers, acetic acid, 1,4-dimethoxy-2,3-butanediol and 1,3-dimethoxy-2-propanol, were proposed to identify patients with giardiasis; these compounds were not present in the patients without the parasite. Multivariate analysis revealed that principal component 1 separated the stool samples according to the presence of infection by *G. duodenalis* despite the inter-individual variability in biological specimens such as faeces.

Keywords Biomarkers · Volatile compounds · *Giardia duodenalis* · Gas chromatography · Chronic diarrhoea · Persistent diarrhoea

Introduction

In children, prolonged episodes of diarrhoea are characterised by an increased frequency, a reduced consistency and an increased volume of bowel movements that do not improve and that last more than 2 weeks; these episodes are considered as chronic and/or persistent diarrhoea (Keating 2005; Baran 2014). The concepts of "persistent diarrhoea" and "chronic diarrhoea" are different. Persistent diarrhoea is an episode of diarrhoea with an infectious aetiology that develops acutely but lasts longer than 14 days (Mathai et al. 2011), whereas chronic diarrhoea is defined as diarrhoea that lasts more than 14 days and is not exclusively associated with an infectious cause (Lee et al. 2012). Therefore, persistent diarrhoea is included in a large overall category called chronic diarrhoea syndrome. The characteristics of the stools and their accompanying symptoms vary according to the pathophysiology of the diarrhoea (osmotic, secretory, steatotic), its impact on the nutritional status of the child and, sometimes, its aetiology (Baran 2014). In this context, one of the most common protozoans associated with persistent diarrhoea is *Giardia duodenalis*, which has been reported in some studies to have a prevalence of 66.4% in patients presenting with this symptom (McHardy et al. 2014).

The detection of *G. duodenalis* by direct immunofluorescence has a sensitivity that ranges between 99 and 100%, with a specificity of 100% compared with conventional techniques (66% for conventional microscopy and 93% for conventional trichrome staining) (García et al. 1992). Current methodologies for the diagnosis of diseases have become diversified, and the use of immunological-based assays and molecular methods has increased (Parčina et al. 2018; Hooshyar et al. 2019). The search for specific biomarkers is the current tendency, and in this scientific field, gas chromatography (GC)

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✉ J. M. Ubeda-Ontiveros
jmubeda@us.es

1 Instituto de Ciencias Biomédicas, Facultad de Ciencias, Universidad Autónoma de Chile, Providencia, Región Metropolitana, Chile

2 Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Seville, 41012 Seville, Spain

has been demonstrated to be a reliable tool to determine characteristic volatile organic compounds for a large range of diseases (Shirasu and Touhara 2011). Furthermore, the detection of volatiles at low levels allows the diagnosis of a disease in the early stages of development. Alterations in the volatile profile of various samples (faeces, urine, sweat, breath) reflect the physiological and physiopathological metabolic processes that may take place in the human body. The employment of scents for the detection of diseases has been used from the time of Hippocrates through today; scent is currently used to detect the presence of diseases such as cancer, Alzheimer's disease and Parkinson's disease (Khatib et al. 2014; Wang et al. 2014; Mazzatenta et al. 2015).

Among the diseases able to be diagnosed with gas chromatography, infections are very interesting because the presence of a specific microorganism is commonly associated with a characteristic smell in various organic samples due to microbial metabolism (Ho and Reddy 2011; Bos et al. 2013). For example, faeces from *Clostridium difficile* patients produces 5-methyl-2-furancarboxyaldehyde (Probert et al. 2004) and isocaproic acid (Tait et al. 2014), faeces from patients with *Campylobacter jejuni* present 1-butoxy-2-propanol and 3-methyl furan (Garner et al. 2007), and faeces from patients with *Vibrio cholerae* produces extremely large quantities of dimethyl disulphide and p-menth-1-en-8-ol (Garner et al. 2009). As mentioned above, *G. duodenalis* is one of the most prevalent protozoans causing persistent diarrhoea in children. In general, patients with giardiasis are reported to expel a foul gas with a distinctive foul smell through eructations or flatuses (Mørch et al. 2009). This characteristic smell has been analysed by Bond et al. (2015) in faeces from patients with *G. duodenalis*; results suggested that 2,2,4,4-tetramethyloctane, acetic acid and 2,2,4,6,6-pentamethylheptane were potential biomarkers of giardiasis. In this study, volatile compounds from faeces were extracted by solid phase microextraction (SPME), a sensible technique employed to extract major and minor volatile compounds from samples. The aim of this study was to detect volatile biomarkers of the presence of *G. duodenalis* from the faeces of patients with chronic diarrhoea. The authors would like to note that the aim was not to propose a direct detection method for *G. duodenalis* with gas chromatography, but rather to detect molecules that are markers of the presence of *Giardia* in faeces to employ these molecules as targets to develop simple diagnostic methods, i.e. colorimetric methods, in the future. For this purpose, we employed the following extraction technique, which is used for the extraction of volatile compounds that are found in samples in large quantities: static headspace extraction followed by gas chromatography coupled to mass spectrometry (HS/GC/MS). The detection of biomarkers found in high amounts in faeces would indicate them as possible targets for the development of more cost effective detection methods for this parasite. Additionally, from a technical point of view, the HS extraction method is cheaper than the SPME technique and prolongs the durability of the equipment because it introduces less interference.

Material and methods

Samples

Samples of faeces from children between 2 and 6 years old suffering from chronic diarrhoea symptoms (20–30 days with diarrhoea) collected in May 2018 in the Hospital Universitario Virgen de Valme, Seville (Spain) were analysed. The microscopic diagnosis of *Giardia* was carried out for all the samples. Ten samples were from patients with diarrhoea syndrome with a non-parasitic aetiology (NPD), and seven samples were from patients with diarrhoea syndrome with *Giardia* presence (GD). Among them, eight samples were from males and 9 were from females. The samples were collected in standard plastic faeces sample receptacles and frozen on the same day of collection for 1 week at − 20 °C until analysis.

Reagents and standards

For the semi-quantification of volatile compounds, 4-methyl-2-pentanol was used as an internal standard supplied by Merck (Darmstadt, Germany) and sodium chloride (NaCl) purchased from Sigma-Aldrich (Madrid, Spain). The Kovats index was calculated using a solution of n-alkanes (C6-C30) from Merck ((Darmstadt, Germany).

Extraction and analysis of volatile compounds

The extraction method employed was static headspace (HS). This method was carried out placing 3.5 g of faeces in a 20-mL vial to which 3.5 mL of a NaCl-distilled water mixture (20%) was added to force the migration of the volatile compounds to the headspace. Next, 10 μL of 4-methyl-2-pentanol (0.75 mg L^{-1}) was added as an internal standard. Finally, the vial was closed with an aluminium screw cap with a silicone septum/PETF (VWR International Eurolab S.L., Barcelona, Spain) and placed in the autosampler.

Analyses were carried out in a Thermo Scientific Gas Chromatograph coupled to a triple quadrupole mass spectrometer (TSQ8000-Thermo Scientific) equipped with an autosampler and automatic injector. The sample in the vial was incubated for 30 min at a temperature of 50 °C with continuous stirring while heating. At the end of this time, 800 μL of gas was taken from the headspace and injected into the gas chromatograph using a 1-mL syringe of heated capacity (50 °C) in split mode 10:1. The injection temperature was 250 °C, and the capillary column employed was a ZB-WAXplus column (30 m × 0.25 mm, 0.25-μm thickness, Zebron). The carrier gas was helium at a constant flow of 1 mL/min. The temperature of the oven started at 40 °C, was maintained for 1 min and then rose to 100 °C at a rate of 2.5 °C/min and then up to 200 °C at a rate of 15 °C/min.

The temperature of the transfer line was maintained at 240 °C. The acquisition of ionization spectrum data was in SCAN mode (30–300 amu) and recorded at 70 eV of energy.

Data analysis

Data processing was carried out with Xcalibur ™ software (Thermo Fischer Scientific). The tentative identification of the compounds was carried out with the comparison of the mass spectra obtained from each molecule with the reference spectra of the software library of NIST 98 software. In addition, a series of standard alkanes (C7-C30) was injected to calculate the Kovats indexes according to the retention times. The Kovats indexes of volatile compounds were compared with those found in the literature to support identification. The relative abundance of each compound was calculated with respect to the internal standard 4-methyl-2-pentanol.

Statistical analysis

The data obtained were subjected to multivariate analysis performing principal components analysis (PCA) with SPSS Software (IBM, Barcelona, Spain).

Results and discussion

The extraction of volatile compounds from faeces was performed using the static headspace extraction method (HS). This method is specifically indicated for the analysis of major volatiles in samples, namely, those with high concentrations. As shown in Table 1, a total of 25 volatile compounds were determined, of which 7 were esters, 6 were alcohols, 6 were terpenes, 2 were sulphur compounds and one each was amide, acid and aldehyde. Esters were the largest group of volatile compounds. In this study, these compounds were found in only 2 of the 10 patients with diarrhoea syndrome of non-parasitic aetiology (NPD); however, in all patients with *Giardia* infection, there was at least one ester in the analysed stool. Esters of short-chain fatty acids, such as acetate, butanoate, pentanoate, hexanoate and methyl heptanoate, ethyl butanoate and propyl butanoate, found in the faeces of patients are compounds that usually have a fruity aroma. These compounds were mainly found in patients with giardiasis. This result agrees with the results of Bond et al. (2015), who described esters as the major chemical volatile group present in faeces of patients with *Giardia* infection; however, they did not find significant differences between the giardiasis group and the control group. In contrast with our results, Bond et al. (2015) found significant differences in the amounts of propyl propanoate and ethyl butanoate between patients with giardiasis and the control group, finding more of both in the patients in the control group. Moreover, Garner et al. (2007) observed a large amount of esters in the faeces of patients with irritable bowel syndrome. This increased presence of esters may be due to their synthesis from short-chain fatty acids by the enzyme acyl CoA; a greater number of short-chain fatty acids have been described in various intestinal diseases (Garner et al. 2007; Di Cagno et al. 2009; Del Chierico et al. 2017).

Two sulphur compounds were found, dimethyl disulphide and dimethyl trisulphide, of which the aromatic descriptions are onion, cooked or rotten cabbage, pungent, sulphurous, fishy, rank and nauseating (Moore et al. 1987; Flavornet 2018). These compounds were found in the headspace of all the samples analysed from the faeces of patients with diarrhoea with a non-parasitic aetiology and were not found in any of the samples of patients with giardiasis (GD) (Fig. 1). Dimethyl disulphide and trisulphide are produced by the metabolism of methionine by various microorganisms present in the intestinal flora because this amino acid is the precursor for its formation (Kadota and Ishida 1972). These compounds usually appear in faeces and are known for their characteristic odour (Moore et al. 1987; Suarez et al. 1998). Both are formed by the oxidation of methyl mercaptan and, in the case of dimethyl trisulphide, also by the oxidation of hydrogen sulphide (Zahn et al. 2001). These sulphur compounds have been described by other authors as biomarkers in diverse diseases such as colorectal carcinoma and *C. jejuni* and *V. cholera* infections (Garner et al. 2007, 2009; Batty et al. 2015). However, for the synthesis of methionine by *Giardia* spp., the presence of L-cysteine is necessary because this amino acid is essential for this parasite to survive (Lujan and Nash 1994). Therefore, it could be possible that in the case of patients with diarrhoea with a non-parasitic aetiology, these compounds are normally produced in large quantities by the intestinal microbiota. However, in the case of patients with giardiasis, the formation of these compounds may be impeded by the high demand for their precursor (L-cysteine) by the parasite. On this basis, the presence of minor amounts of dimethyl disulphide and trimethyl disulphide in the faeces of patients with giardiasis would be justified in part and, therefore, not detectable in the sample taken from the headspace and subjected to HS/GC/MS, which is able to detect major volatile compounds. Therefore, the absence of these compounds in the headspace of stool samples could be an indicator of the presence of *Giardia* or another microorganism whose metabolism is strictly dependent on the amino acid cysteine. This partially coincides with the results of Bond et al. (2015), who found that dimethyl trisulphide was present in decreased amounts in patients with the parasite by extracting the volatile compounds from the faeces headspace of patients with and

Table 1 Volatile compounds determined in the faeces from patients with chronic diarrhoea, with non-parasitic aetiology (NPD) and patients with giardiasis (GD). Results are expressed in relative areas in reference to 4-methyl-2-pentanol. *ID*, reliability of identification; *LRI*, Linear Retention Index

			Patients with non-parasitic diarrhoea										Patients with giardiasis						
			NPD1	NPD2	NPD3	NPD4	NPD5	NPD6	NPD7	NPD8	NPD9	NPD10	GD1	GD2	GD3	GD4	GD5	GD6	GD7
LRI	ID																		
		Esters																	
782	A	Methyl acetate	–	–	–	–	–	–	–	–	–	–	–	–	1347	–	7471	4939	–
998	A	Methyl butanoate	–	–	–	–	–	340	–	–	–	–	–	63	268	641	497	343	–
1003	A	Ethyl butanoate	–	–	–	–	–	40	–	–	–	–	–	–	106	–	–	–	–
1103	A	Methyl pentanoate	–	–	–	–	–	151	–	15	–	–	–	–	–	227	920	653	64
1079	A	Propyl butanoate	–	–	–	–	–	37	–	–	–	–	–	–	–	–	–	–	–
1207	A	Methyl hexanoate	–	–	–	–	–	–	–	–	–	–	28	–	–	–	94	107	–
1308	A	Methyl heptanoate	–	–	–	–	–	–	–	–	–	–	16	–	–	–	–	–	–
		Alcohols																	
961	B	1,3-Dimethoxy-2-propanol	–	–	–	–	–	–	–	–	–	–	725	561	–	326	939	579	110
1023	A	Propanol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	239
1099	A	Isobutanol	–	–	–	–	–	20	–	–	–	–	–	–	–	–	–	–	–
1134	A	Butanol	–	–	–	14	–	90	15	–	–	–	–	–	–	–	–	–	–
1247	A	Pentanol	–	–	–	–	–	19	–	–	–	–	–	–	–	–	–	–	–
1287	B	1,4-Dimethoxy-2,3-Butanediol	–	–	–	–	–	–	–	–	–	–	113	55	23	44	94	54	69
1620	A	p-Cresol	–	90	68	–	103	–	–	40	–	32	–	–	–	–	–	–	–
		Sulphur compounds																	
1069	A	Dimethyl disulphide	3336	59	99	1201	47	389	38	355	18	470	–	–	–	–	–	–	–
1366	A	Dimethyl trisulphide	147	–	–	19	–	45	–	15	–	19	–	–	–	–	–	–	–
		Terpenes																	
1061	B	β-Terpinene	–	–	–	127	–	–	–	–	–	–	–	–	–	–	–	–	–
1072	B	β-Fellandrene	–	–	–	–	–	–	–	–	–	9	–	–	–	–	–	–	–
1113	A	β-Pinene	–	23	173	16	–	–	–	–	12	–	–	–	–	–	–	–	75
1121	A	δ-Limonene	207	61	–	20	–	–	–	–	32	–	–	9	–	22	9	–	65
1130	A	δ-3-carene	–	18	–	91	–	–	–	–	39	12	–	–	–	–	–	–	78
1260	A	p-Cymene	–	–	–	–	–	11	–	–	–	–	–	–	–	–	–	–	–
		Acids																	
1457	A	Acetic acid	–	–	–	–	–	–	–	–	–	–	376	213	89	139	143	111	309
		Miscelaneous																	
905	A	2-Hidroxypropanamide	–	–	–	315	–	–	–	–	–	–	250	427	280	534	335	279	879
1089	A	Hexanal	–	–	–	–	–	–	37	–	–	–	–	–	–	–	–	–	–

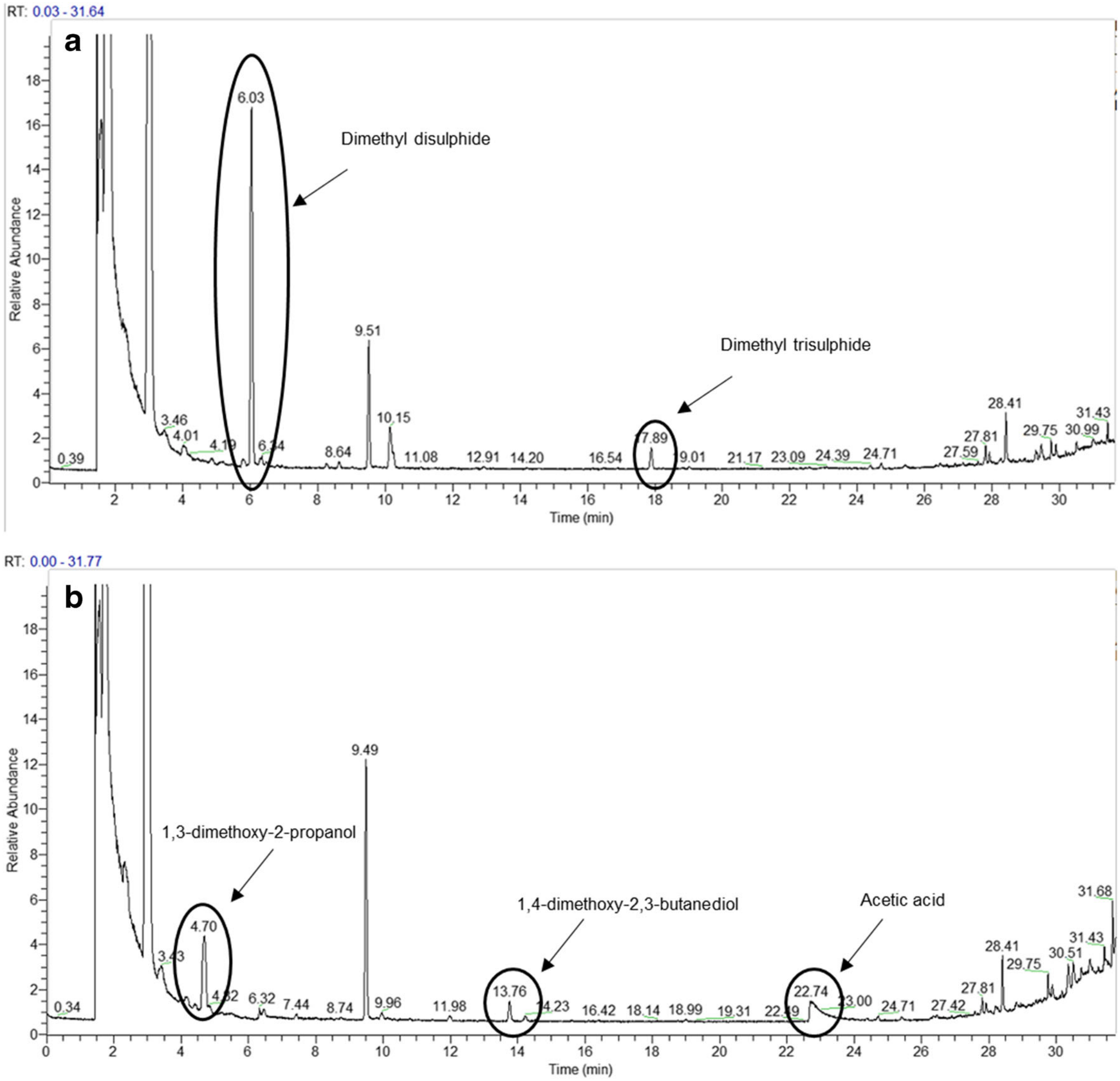

Fig. 1 Chromatogram from patient with diarrheic syndrome of non-parasitic aetiology (NPD) (**a**) and from patient with diarrheic syndrome with *Giardia* presence (GD) (**b**)

without giardiasis with the solid phase microextraction technique. However, these differences were relatively small and did not indicate differences with respect to dimethyl disulphide. Therefore, the presence of these sulphur compounds in the headspace of the faeces samples could be related to the absence of *G. duodenalis* in the host.

With respect to the group of alcohols, interesting trends for two alcohols were observed. The first, 1,3-dimethoxy-2-propanol, only appeared in patients with giardiasis, specifically in 6 of the 7 GD patients. This alcohol is the major component of the leaf gel in *Aloe vacillans*, a succulent plant that belongs to the Aloe family that is very abundant in the desert in Arabia Saudi. This gel has been traditionally used for the natural treatment of gastrointestinal disturbance due to its antimicrobial activity, and its major volatile compound is 1,3-dimethoxy-2-propanol (Alrumman 2018). This volatile organic compound may be secreted by the parasite to eliminate the protective microbiota in the intestine to avoid competition for metabolites. The second compound, 1,4-dimethoxy-2,3-butanediol, also appeared in only patients with giardiasis, and in this case, in 100% of the patients. Regarding this compound, there is only one clear bibliographic reference that indicates that this compound is a possible marker of breast cancer; it was analysed in samples of exhaled air from patients with the diagnosed disease and air from control patients (Wang et al. 2014). In that work, they attributed the presence of this alcohol to oxidative stress (Knight 1998). To the best of our knowledge, these compounds have never been described in faeces, and they could be compounds produced by the particular metabolism of this parasite. Therefore, 1,3-dimethoxy-2-propanol and 1,4-dimethoxy-2,3-butanediol could act similarly to some long-chain aliphatic alcohols that exert an inhibitory effect on the growth of other microorganisms, creating an ecological niche for *Giardia* spp. This effect has been observed with a range of bacteria and different long-chain aliphatic alcohols (Elgaali et al. 2002).

In contrast, the terpene compound group did not show a notable trend. Terpenes are compounds that, in most matrices, usually have an important contribution to the global aroma because their threshold of perception is very low and they are responsible for citrus and green notes. Nevertheless, in faeces, the other volatile compounds present do not allow the terpene compounds to reveal their odour. Several of the terpene compounds found in this study have been observed in the faeces of patients with ulcerative colitis and those with *C. jejuni* and *C. difficile* infections (Garner et al. 2007). Furthermore, in some diseases, such as *C. jejuni* infection, terpenes have been highlighted as potential biomarkers (Probert et al. 2004).

Although acids are compounds commonly found in faeces, in this study, only acetic acid was present in the headspace of the analysed samples. This volatile compound is described as "pungent" and has an aroma similar to vinegar (Callejón et al. 2008). Specifically, it was detected in the stool samples from 100% of the patients diagnosed with giardiasis and in none of the samples from control patients (Fig. 1). Short-chain fatty acids, such as acetic acid, are usually the products of the metabolism of non-digestible carbohydrates such as fibre, proteins and peptides by colonic bacteria (Backhed et al. 2005). The amount of short-chain fatty acids present in the stool may be an indication of certain diseases related to the intestinal tract. If they are found in high amounts, they may indicate celiac disease, non-alcoholic fatty liver disease or infection with *C. difficile* (Garner et al. 2007; Di Cagno et al. 2009; Del Chierico et al. 2017). Our results coincide with those reported by Bond et al. (2015), who recently described acetic acid as a possible marker of *Giardia* infection. They analysed the volatile compounds in the headspace of faeces from 16 patients with *Giardia* infection and 17 controls; in 12 of the infected patients, they found this volatile, and in the uninfected group, they found it in only one sample. Therefore, the presence of acetic acid could be a biomarker for the presence of this parasite in the host.

The presence of certain compounds, such as acetic acid, 1,3-dimethoxy-2-propanol and 1,4-dimethoxy-2,3-butanediol, in the faeces of the patients with *Giardia* and their absence in the controls could indicate that the release of these compounds may alter the composition of the microbiota, decreasing the parasite competition for metabolites and guaranteeing *Giardia* spp. survival; this indicates an interaction of the parasite with the microbiota of the intestine. The effect of the compounds released from *Giardia* was observed by Beatty et al. (2017) who observed increased levels of inflammatory markers in humanised germ-free mice that were reconstituted with human intestinal microbes that had been exposed to *Giardia* products but not the living parasite (Beatty et al. 2017). They also showed that these biofilms that were altered by the action of the parasite were capable of inducing intestinal permeability due to microbial community disruption caused by *Giardia* secretory proteases.

Another remarkable volatile compound is p-cresol (4-methylphenol). This aromatic compound is described as medicinal, phenolic and smoky (Flavornet 2018). The results showed that this compound was only found in the faeces of patients with non-parasitic aetiologies; specifically, it was identified in 5 of the 10 NPD samples analysed. P-cresol is a volatile phenol that is toxic to humans; in fact, it is a biomarker for assessing the health of the small intestine in humans (Birkett et al. 1996; Muir et al. 2004; De Preter et al. 2006). This phenol has been described as a biomarker of infections with *C. difficile* in stool (Tait et al. 2014). Therefore, this compound may indicate the presence of some kind of bacteria in these patients.

Finally, a principal components analysis (PCA) was carried out with all the volatile compounds (25 variables) to allow a reduction in the dimension and achieve a better understanding of the obtained results. All the samples were included in the multivariate analysis. The analysis of the data resulted in 9 principal components (PCs) with an Eigenvalue greater than 1, which explained 93.5% of the total variance (Table 2). This great variability in the analysed samples was expected since the volatile composition of the faeces depends on many variables, such as nutrition, individual metabolism, the composition of the microbiota of the host and medications, in addition to the pathogenic microorganism. However, despite this great

Table 2 Eigenvalues and percentage of explanation of the main axes

Component	Eigenvalues		
	Total	% of variance	% cumulative variance
1	6.054	24.215	24.215
2	5.108	20.431	44.646
3	2.870	11.479	56.125
4	2.685	10.740	66.865
5	1.884	7.536	74.401
6	1.430	5.718	80.120
7	1.252	5.006	85.126
8	1.103	4.411	89.537
9	1.005	4.022	93.558
10	0.595	2.379	95.937
11	0.490	1.958	97.895
12	0.225	0.902	98.797
13	0.165	0.661	99.458
14	0.089	0.355	99.813
15	0.046	0.182	99.995
16	0.001	0.005	100.000

variability, the multivariate analysis separated the samples according to the presence of infection by *G. duodenalis.* Despite the varying characteristics of this type of sample, PC1 separated the stool samples according to the presence of infection by *G. duodenalis* (Fig. 2). Figure 2a shows the distribution of the analysed samples (scores) represented in principal component 1 (PC1) and principal component 2 (PC2), which represented 24.2% and 20.4% of the variance, respectively (44.6% of the cumulative variance). Thus, positive values in PC1 would indicate the presence of the parasite in the host and are grouped on the right side of the graph, while patients who had chronic diarrhoea but who did not have the parasite in their faeces had negative values in PC1 and are grouped on the left side of the graph. Figure 2b shows the variables and how they relate to the samples (loadings). In Fig. 2, considering that PC1 seemed to separate according to the presence of *G. duodenalis* infection, the relationship between the presence of some compounds and the pathological situation of the patient can be observed. In this sense, the volatile alcohols 1,3-dimethoxy-2-propanol and 1,4-dimethoxy-2,3-butanediol had a very important weight in the separation of patients with giardiasis, showing high loadings of 0.809 and 0.781, respectively (Table 2). Acetic acid also presented high loading (0.682), reaffirming the correlation between the presence of this compound and the parasite in the host.

It is worth mentioning that a patient without *G. duodenalis* was placed correctly on the left side of the graph as a non-infected patient; however, this patient was separated from the rest of the group of patients with chronic diarrhoea without the parasite. An explanation is that this patient had 3 volatile

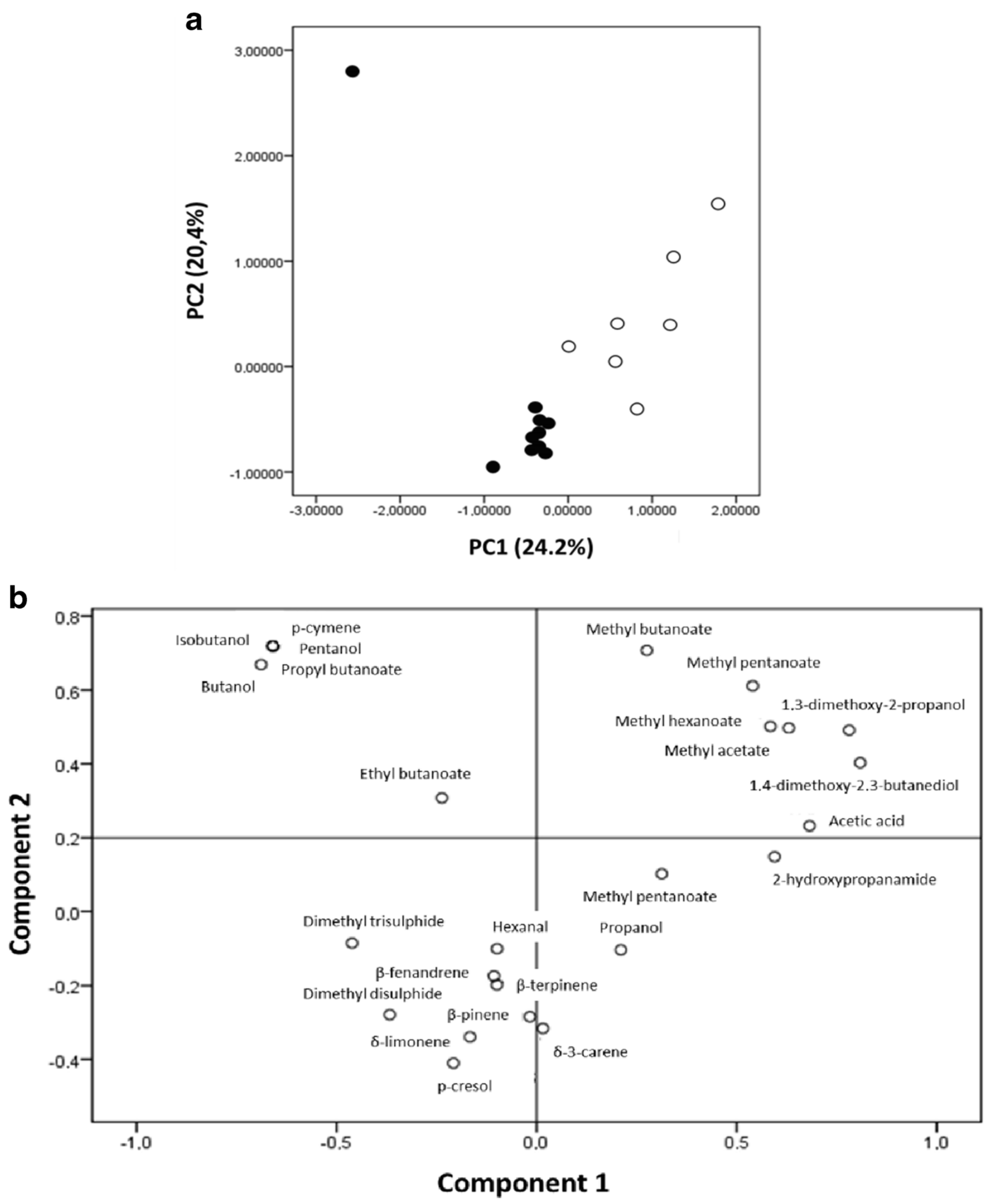

Fig. 2 Data scores (**a**) and loadings (**b**) biplot on the plane made up of the first two principal components (PC1 against PC2). In black are NPD patients and in white DG patients

compounds that were not present in the faeces of any other patient in this study: isobutanol, pentanol and p-cymene. This could be due to the existence of another gastrointestinal pathology different from that of the rest of patients. This fact enhances the importance of the study of secondary metabolites as volatile compounds for the detection of diseases since these 3 compounds could be biomarkers of another pathological condition.

Conclusions

The static headspace extraction method used for the analysis of major volatile compounds in faeces has been shown to have sufficient sensitivity and efficacy to identify volatile compounds that are able to discriminate between patients with and without giardiasis. According to these results, the volatile compound profiles of the faeces of patients infected with *Giardia* were different from those of patients with diarrhoea but without *Giardia*. The use of chromatographic techniques allows the determination of some volatile compounds, such as 1,4-dimethoxy-2,3-butanediol, 1,3-dimethoxy-2-propanol and acetic acid, as possible biomarkers of the presence of *Giardia* in the faeces. Multivariate analysis revealed that despite the inter-individual variability in a biological sample such as faeces, the prominent biomarkers are present in high enough amounts to discriminate between those infected by *G. duodenalis* and those without the parasite.

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Compliance with ethical standards

The experiment was approved by the committee: Comité de ética de investigación de Sevilla Sur del Hospital Universitario de Valme (No. 1569-M1-17).

Informed consent Informed consent was obtained from all individual participants included in the study (parents of patients).

Conflict of interest The authors declare that they have no conflict interest.

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