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Determination of low-molecular mass aldehydes by automated headspace solid-phase microextraction with in-fibre derivatisation

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Abstract

Headspace solid-phase microextraction (HS-SPME) analysis of low-molecular mass (C_1–C_{10}) aldehydes in aqueous solutions was investigated, using pentafluorophenylhydrazine (PFPH) and *o*-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) as in-fibre derivatisation reagents. Analysis of the derivatives was achieved, using GC–flame ionisation detection (FID). A comparison of the two reagents showed that PFBHA was superior to PFPH under the investigated conditions. Fundamental studies of the PFBHA and PFPH reactions showed that the kinetics of the process was limited by the mass transport rate of the analytes to the fibre. The developed PFBHA method gave detection limits in the low to sub-microgram per litre range for most of the aldehydes tested. The method was applied successfully to the analysis of particleboard, wine and fish samples.

Keywords: In-fibre derivatisation; Solid-phase microextraction; Aldehydes; Pentafluorophenylhydrazine; Pentafluorobenzylhydroxylamine hydrochloride; Automation

1. Introduction

Interest in the analysis of low-molecular mass aldehydes has increased significantly in recent years. These and other carbonyl compounds exist naturally in the environment as a result of phenomena, such as the photodegradation of dissolved organic matter, microbiological processes and the photo-oxidation of hydrocarbons in the atmosphere [1,2]. Anthropogenic sources, such as combustion of fossil fuels and chemical manufacturing are also significant [1,3,4]. Indoor air environments often contain elevated levels of formaldehyde and other aldehydes resulting from their use or presence in many building materials and other products [5,6]. Disinfection of drinking water using ozonation creates a number of byproducts, including aldehydes through reaction with organic matter [7]. The primary aldehydes produced through this process are formaldehyde, acetaldehyde, glyoxal and methyl glyoxal [7,8].

Health concerns have played a significant role in generating interest in the sensitive determination of these aldehydes. Formaldehyde and acetaldehyde are known animal carcinogens and have been listed as probable or suspected human carcinogens by the US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) respectively [9,10]. The US National Institute for Occupational Safety and Health (NIOSH) has indicated that a number of low molecular weight aldehydes, including propanal, butanal, pentanal and glyoxal possess mutagenic properties [9].

Because of their high volatility and reactivity it is usually necessary to derivatise these aldehydes prior to analysis in order to achieve satisfactory recovery and sensitivity. Some example reagents include, 2,4-dinitrophenylhydrazine (DNPH) [11,12], which is generally used in conjunction with HPLC analysis and *o*-2,3,4,5,6-(pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) in conjunction with GC–electron-capture detection (ECD) or GC–MS to determine the oxime products [11,13,14]. A number of other reagents have also been used [7,11,15,16]. These methods of analysis have traditionally required substantial sample preparation since

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the derivative products need to be isolated prior to chromatography through some kind of solvent extraction procedure.

Solid-phase microextraction (SPME) is a solvent free extraction and pre-concentration technique [17]. The technique uses a modified syringe-like device containing a polymeric extraction phase that allows considerably faster sample preparation than is generally possible by traditional methods [18,19]. Several papers have appeared using derivatisation of aldehydes in conjunction with SPME. PFBHA derivatisation in water samples followed by extraction of the derivative products by headspace SPME has been reported by Bao et al. [2] and more recently by Cancho et al., who compared this methodology with EPA method 556 which uses PFBHA with solvent extraction [7]. This approach has also been applied to the determination of aldehydes in alcoholic beverages [20]. An alternative means of performing the derivatisation is to add the derivatisation reagent to the SPME fibre coating and then expose this to the headspace of the sample. In this instance the derivatisation reaction occurs in the fibre coating. Using PFBHA, this technique has been applied successfully to the determination of formaldehyde [21,22], pentanal [23] and glutaraldehyde [24] in air. For liquid samples, the technique has been applied to the determination of formaldehyde, propanal, butanal and pentanal in water [25] and a variety of aldehydes in beer [26]. Stashenko et al. have reported the use of an alternative reagent, namely pentafluorophenylhydrazine (PFPH), which they have used for in-fibre derivatisation analysis of aldehydes in vegetable oils [27,28]. For both derivatisation reagents poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB) SPME fibres were used.

This work aimed to investigate some fundamental aspects relating to use of in-fibre derivatisation for C_1–C_{10} aldehydes, issues relating to automation and compare the relative merits of the PFBHA and PFPH reagents.

2. Experimental

2.1. Chemicals and reagents

Acetaldehyde (>99.5%), propanal (97%), butanal (99%), pentanal (97%), 2-methylpentanal (98%), hexanal (98%), heptanal (95%), octanal (99%), nonanal (95%), decanal (95%), glyoxal (40% in water), methylglyoxal (40% in water), PFBHA (>98%) and PFPH (97%) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Formaldehyde (37% in water) and sodium chloride was purchased from EM Science (Gibbstown, NJ USA). Methanol was purchased from Fisher Scientific Canada (Nepean, Canada). Water purified from a Barnstead ultrapure water system (Dubuque, IA, USA) was used throughout. PDMS–DVB (65 μm) SPME fibres were purchased from Supelco (Bellefonte, PA, USA). All gases were supplied by Praxair (Kitchener, Canada) and were of ultra high purity.

The dry white wine, fish and particleboard samples were obtained from a local liquor store, supermarket and hardware store, respectively.

Individual stock standard solutions for each aldehyde were prepared in HPLC grade methanol at a concentration of 2000 mg/L and stored at 4 °C. A working standard solution containing each aldehyde at a concentration of 1000 μg/L was prepared from the stock standards by appropriate dilution with saturated sodium chloride solution in water.

2.2. Gas chromatographic analysis

Gas chromatography was performed, using a Varian (Mississauga, Canada) 3800 gas chromatograph equipped with a flame ionisation detection (FID) system with instrument control and data collection provided by Star Chromatography Workstation software version 5.51. GC–MS analyses were performed, using a second Varian 3800 instrument coupled to a Varian Saturn 2000 MS detector. Compound identifications were made using spectral libraries supplied with the software. Automation of the procedure was achieved using a CTC CombiPal autosampler (Zwingen, Switzerland), which was programmed using CycleComposer software version 1.4.0 and equipped with sample trays, a temperature controlled agitator tray and a fibre-conditioning device. Sample vials had a total volume of 10 mL and used magnetic crimp caps with PFTE coated silicone septa (Microliter Analytical Supplies, Suwanee, GA, USA). Separation was performed using a 30 m × 0.25 mm i.d., 0.25 μm Rtx-5MS fused silica column from Restek (Bellefonte, PA, USA). For analysis using PFPH as derivatisation reagent, the GC oven program was as specified by Stashenko et al. [27] and the injector temperature used was 260 °C. While using PFBHA as derivatisation reagent, the GC temperature program was 50 °C (1-min hold) to 220 °C at 4 °C/min, then to 250 °C at 20 °C/min (10-min hold) as specified in EPA method 556 [14]. In this case, the injector temperature used was 250 °C. Helium was used as carrier gas in both methods. For the PFPH method, the flow rate was 1 mL/min, whereas for the PFBHA method a constant pressure of 15 psi was applied. For experiments examining the reproducibility of derivatisation reagent loading on the fibre an injector split ratio of 20:1 (PFPH) or 50:1 (PFBHA) was used. The amount of PFBHA loaded on the fibre was determined by comparing peak areas with those of a calibration curve using a split ratio of 250:1. The calibration points were established manually by dispensing ≤1 μL of a PFBHA standard solution in methanol onto the surface of the fibre coating, letting the methanol dry and then injecting into the GC.

FID conditions for both methods were identical. An operating temperature of 300 °C was used with gas flow rates for hydrogen, air and nitrogen of 30, 300 and 25 mL/min, respectively.

The autosampler parameters used for both derivatisation reagents except where otherwise specified are given in Table 1. To complete one analysis cycle the fibre was first

Table 1
Autosampler method parameters

Parameter	Value
Agitator temperature	50 °C
Pre-extraction equilibration time in agitator (for both loading and extraction steps)	1 min
Pre-extraction agitator speed	750 rpm (programmed to spin in the following cycle: 5 s clockwise, 2 s pause, 5 s anti-clockwise, 2 s pause)
Loading time	5 min
Loading agitation speed	250 rpm
Extraction time	5 min
Extraction agitation speed	500 rpm
Desorption time	5 min
Fibre conditioning temperature	260 °C
Bake-out time in conditioning unit	1 min

desorbed in the conditioning device. The vial containing the derivatisation reagent was transferred to the agitator and allowed to equilibrate to the sampling temperature. Then, the fibre was exposed to the headspace of this vial. A similar protocol was followed for the extraction of aldehydes from the sample vial, although the agitator speed used was faster. Finally, the fibre was desorbed in the GC injection port and the chromatographic run commenced.

Both derivatisation methods produce two geometrical isomers for analytes containing a single aldehyde group. Formaldehyde was an exception as it is symmetric, and therefore, only forms a single derivative. In many cases, these two isomers were resolved on the chromatographic column. Since there was a large difference in peak area between the two isomers of each aldehyde, the larger of the peaks was used for quantitation, using PFPH. For PFBHA the peak areas were generally similar, and therefore, the sum of both peak areas was used for quantification unless otherwise specified.

2.3. Sample preparation

Sawdust from the particleboard was generated from an off-cut of this material and collected in a small beaker. From this, 0.0200 ± 0.0005 g portions were weighed into 10 mL headspace vials followed by 2.0 mL of saturated sodium chloride solution. After capping each vial, the samples were left to equilibrate at ambient temperature for over 1 h before analysis.

The white wine sample (ethanol content 11.5%) was diluted to a concentration of 5% (v/v) with saturated sodium chloride solution. From this, 2.0 mL portions of the solution were analysed in 10 mL vials against standards that were prepared to contain the same concentration of ethanol as in the diluted sample (0.6%, v/v).

To prepare the fish (Pollock) samples, 1.000 ± 0.0100 g of finely chopped raw meat was added to a series of vials and mixed with 2.0 mL saturated sodium chloride solution. The samples were prepared by agitating at 50 °C for over 30 min prior to analysis.

3. Results and discussion

3.1. Initial experiments

In previous studies with PFPH as in-fibre derivatisation reagent, 0.1 mM PFPH was used, but this concentration required a 60 min loading time [27]. To obtain an acceptable loading on the fibre in a shorter time 10 mM PFPH was used. PFBHA was loaded on the fibre from the headspace of a 17 mg/mL solution as has been reported in previous papers using this reagent [21–25]. Loading temperature was investigated between 30 and 60 °C. PFPH loading increased to a maximum at 50 °C and PFBHA increased progressively across this temperature range. From these experiments 50 °C was chosen as the loading and extraction temperature for this study. Using these conditions, a 5-min loading time resulted in an acceptable fibre loading of each reagent.

Initial experiments performed, using PFPH, gave a much larger variability in the loaded amount if 2.0 mL of derivatisation reagent solution and a 500 rpm stirrer speed were used. This was attributed to liquid from the vial sometimes splashing the fibre during sampling. Using a 250 rpm stirrer speed with 1.0 mL of the reagent in the vial solved this problem. These conditions were therefore used for reagent loading throughout the remainder of the project. A study of 50 fibre-loading cycles from the same 10 mM PFPH solution vial showed that the amount adsorbed was essentially unchanged over this period (R.S.D. = 5%). In contrast, PFBHA showed a slow steady decline in loading over this number of cycles equating to an approximately 30% decline in peak area. This restricts the number of injection cycles that can be performed from one vial of the PFBHA reagent. As a result no more than 32 injection cycles were performed using the same PFBHA solution. Loading the fibre from a vial containing solid PFBHA to improve the reproducibility was unsuccessful.

MS was used to identify the large derivatisation reagent peak observed in each system. A positive identification was obtained for PFPH. However, for the PFBHA system the peak did not give a spectrum that was consistent with this compound. The reference mass spectrum is dominated by a peak at *m*/*z* 181 corresponding to the pentafluorobenzyl group, whereas in this work a spectrum with the largest peak at *m*/*z* 394 with *m*/*z* 214 second was observed. The latter peak is likely to be generated by unfragmented PFBHA. Further investigation showed that mass spectra obtained at the edges of the PFBHA peak closely matched the reference spectrum, indicating that the high concentration of the reagent on the fibre coating overloads the MS preventing it from effectively fragmenting all the PFBHA molecules. The *m*/*z* 394 peak is suspected to be the result of free pentafluorobenzyl groups combining with unfragmented PFBHA molecules in the MS.

Extraction profiles with PFPH and PFBHA were studied from 1 to 60 min. Fig. 1 shows example profiles for formaldehyde, acetaldehyde, propanal and butanal, using the PFBHA reagent. With both reagents the rate of derivative formation was initially fast but slowed considerably over the tested

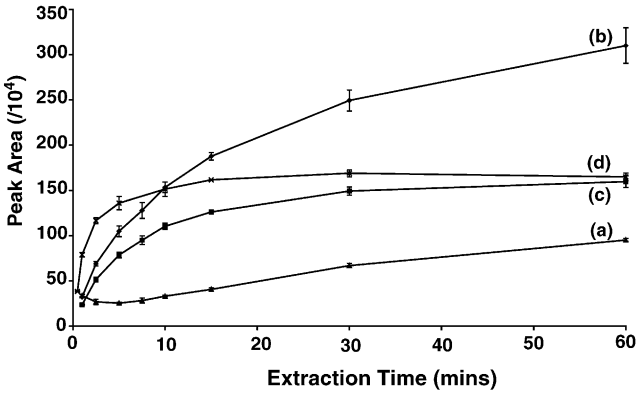

Fig. 1. Extraction profiles for 100 μg/L solutions of (a) formaldehyde, (b) acetaldehyde, (c) propanal and (d) butanal using the PFBHA in-fibre derivatisation procedure. For other conditions see text.

period, for some aldehydes even reaching a complete plateau indicating that either analytes or reagent has been substantially consumed during the reaction. Formaldehyde was an exception that showed a steadier increase in derivative formation with extraction time over the tested range using both derivatisation reagents. This behaviour is similar to that previously observed by Tsai and Chang [25] with PFBHA. This observation can be explained by its higher affinity towards the water phase, compared to the other aldehydes studied which with the exception of glyoxal and methyl glyoxal are generally considered to be at least two orders of magnitude smaller [29–31]. The initial drop in formaldehyde derivative peak area between 1 and 5 min observed in Fig. 1 is attributed to variation in the amount of formaldehyde contamination present in the system. Using the in-fibre derivatisation approach for glyoxal and methylglyoxal was unsuccessful with both PFPH and PFBHA, with no peak observed for either compound. These compounds were therefore not considered further. This again can be attributed, at least in part, to the higher affinity of these compounds for the aqueous phase [29], combined with the formation of multiple derivatives for these species.

Repeated 60-min extractions from a single vial, containing 100 μg/L of each aldehyde with PFBHA in-fibre derivatisation, showed that at least 90% of each aldehyde in the vial was removed in the first extraction, except for formaldehyde, octanal and nonanal (decanal was not tested in this experiment). Formaldehyde was by far the least extracted aldehyde with approximately 50% remaining for the second extraction. The formaldehyde result is probably again linked to the affinity of this substance for the aqueous phase, which would reduce the rate at which it is transferred from the sample to the fibre. The lower extraction of octanal and nonanal can probably be explained on the basis of the lower vapour pressure over water of these aldehydes. Five minutes was eventually chosen as the extraction time.

To investigate behaviour of the system without the presence of aldehydes, extractions from vials containing only 2.0 mL of saturated sodium chloride solution, were performed. These showed that with increasing extraction time a significant decrease in the derivatisation reagent loading on the fibre was observed for both reagents. For example, the peak area of PFBHA for a 5-min extraction was 55% lower and for a 60-min extraction was 73% lower than the value observed, using 1 min. This behaviour is not surprising since these volatile reagents will re-establish equilibrium with the headspace and sample contained in the vial. Because of the significantly larger volume of the headspace and sample compared to the fibre coating the capacity of the fibre would have to be very large to stop a significant portion of the reagents from shifting to the headspace and sample at equilibrium. As a general note, this behaviour should be taken into consideration when developing in-fibre methods as in certain circumstances it may influence the derivatisation process, although this was not the case for the applications tested in this work. If necessary improvements to the method that could be considered in the future would be to find a way of immobilising the reagent on the coating, a coating that has a higher affinity for the derivatisation reagent or to find an alternative, less volatile reagent for the process.

During the study, background contamination peaks were observed, most notably for formaldehyde, as has previously been noted by a number of other researchers [32]. Using PFBHA typical formaldehyde concentrations observed were approximately 25 μg/L. Concentrations, using PFPH, were significantly higher at approximately 65 μg/L, indicating a higher level of impurity in the derivatisation reagent. To improve the sensitivity and accuracy of the methods at low levels for formaldehyde, further precautions are necessary. EPA method 556 [14] recommends procedures such as the use of a reagent grade water generator with an UV light exposure step or distillation of the reagent water from acidified potassium permanganate. Low-level contamination peaks were observed for all the aldehydes when using PFBHA but not with PFPH, although this was attributed to higher detection limits with the latter reagent.

The use of a 5 min desorption time and 1 min bake-out in the fibre conditioning device at 260 °C gave a carryover of <1% after analysing a 1000 μg/L solution of all the tested aldehydes. A single SPME fibre could be used successfully for more than 100 analysis cycles, using either derivatisation reagent.

3.2. Kinetic experiments

The reaction rate in the fibre can usually be expressed in the form given in Eq. (1) [18]:

$$\frac{\partial[P]_f}{\partial t} = k'[R]_f[A]_f \quad (1)$$

where $[P]_f$, $[R]_f$ and $[A]_f$ are the concentrations of product, derivatisation reagent and analyte in the fibre respectively, and k' is the reaction rate constant. It has been usually assumed that the concentration of the derivatisation reagent

will not change significantly throughout an extraction and also that the process is reproducible for multiple extractions. This is not necessarily so, as demonstrated in preliminary experiments for PFBHA, where the loading of the fibre decreased 30% over the course of 50 injection cycles and over half the reagent was lost to the headspace and sample over the course of a 5 min extraction. As a general comment, other possible problems with an in-fibre derivatisation system include decomposition of the reagent in the fibre coating and changes to the loading behaviour of the coating with repeated use. Competition between analytes for reagent may also be an issue.

These effects could have severe ramifications on the accuracy and precision of the results. However, the rate-determining step of the reaction process is significant in determining how significant these issues could be. There are two possible scenarios, the rate of derivative product formation is dependent on the rate of the derivatisation reaction itself or it is determined by diffusion of the analytes to the reaction site. In the former case, the rate of derivative formation will be particularly sensitive to changes in the concentration of the reagent in the fibre between runs. For the latter case, the reagent concentration is not critical as long as it is in sufficient excess throughout the extraction process.

With these considerations in mind, kinetic experiments were carried out to investigate whether the PFPH and PFBHA are reaction rate or diffusion limited processes. This involved looking at the effect of temperature and agitation speed on the resulting peak areas. The results of these experiments for PFBHA are shown in Fig. 2. The results for PFPH showed similar trends.

From the graphs it is apparent that increasing temperature or agitation speed both increased the amount of aldehyde derivatives formed. Increasing temperature will increase the rate of derivatisation formation through increasing the reaction rate and facilitating faster transfer of analytes to the fibre. Faster agitation can only increase the rate of derivatisation through increasing the mass transfer rate of aldehydes to the fibre coating. For a reaction rate limited process agitation rate should have no effect on derivative formation. There would also be an expectation that the analytes would accumulate in the fibre and underivatised aldehyde peaks would therefore be observed in the subsequent chromatograms (unless extraction was exhaustive). Such peaks were not observed in these experiments. The results, therefore, demonstrate that the reaction rate is fast and that mass transfer of the aldehydes from the sample to the derivatisation reagent in the fibre coating is the rate-determining step of the process. Martos and Pawliszyn [21] have previously investigated the kinetics of the PFBHA reaction for formaldehyde in the gas phase and found that the reaction was the rate-determining step of the process. This indicates that the barrier limiting mass transfer occurs at the sample/headspace interface. The maximum practical agitation speed of 500 rpm was used for the remainder of this work to maximise derivative formation during the sampling period. The agitator unit of the autosampler is capable of rotating at 750 rpm but from previous experience such agitation rates often cause the 24-gauge SPME fibre needle to shear off during extraction. Alternative 23-gauge fibre assemblies that can cope with more vigorous agitation speeds are available, although these are designed for use with a septumless injection system that was unavailable in the laboratory during these experiments.

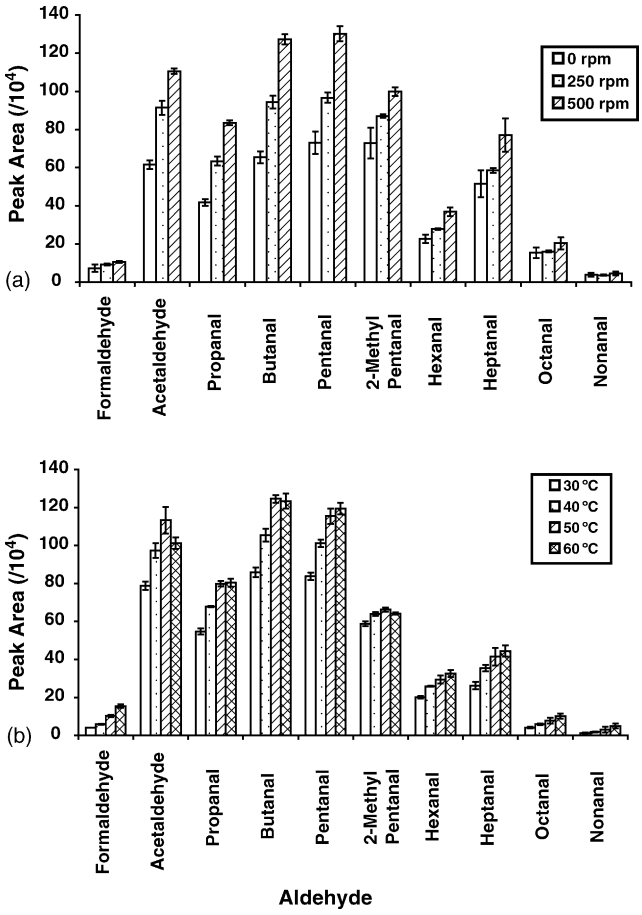

Fig. 2. Chart of aldehyde derivative peak area as a function of (a) agitation speed and (b) temperature using the PFBHA in-fibre derivatisation procedure. Aldehyde concentration 100 μg/L. For other extraction conditions see text.

Another aspect of in-fibre derivatisation that must be considered is the case where there are high levels of one or more compound(s) that can react with the derivatisation reagent in the presence of low levels of other analytes. It is obvious that such a compound, if present in a large enough quantity, will affect the quantification through consumption of the derivatisation reagent. The question is "How much is too much?". The first step was to establish the concentration of derivatisation reagent in the fibre, which was determined to be 0.08 μmol for PFBHA using the chosen conditions. However, since over half of this is desorbed from the fibre coating over the course of the extraction the effective concentration will actually be significantly lower than this for most of the extraction process. It is also unlikely that all of this reagent can be used before the rate of reaction is affected. To study this

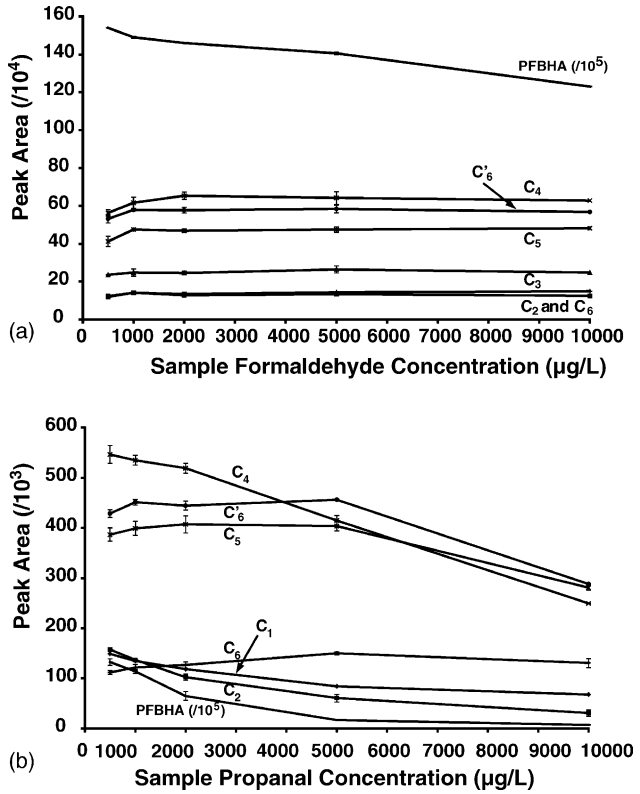

Fig. 3. Effect of large (a) formaldehyde and (b) propanal sample concentration on derivative formation of C_1–C_6 aldehyde derivatives present in the solutions at a concentration of 50 μg/L using the PFBHA method. For other conditions see text (C_6' = 2-methylpentanal).

aspect of the system, the effect of various elevated formaldehyde and propanal concentrations in a solution containing 50 μg/L solutions of other aldehydes was examined. The results are shown in Fig. 3. Formaldehyde concentration does not seem to exert a significant influence on the peak area of the other aldehydes, even when present at a concentration of 10,000 μg/L. Propanal, with its much higher affinity for the headspace and ability to access the fibre coating, causes a dramatic effect on the aldehyde peak areas at and above high microgram per litre concentrations. Interestingly, the lower (C_1–C_4) aldehydes are affected far more strongly and at much lower concentrations of propanal than for the C_5–C_6 aldehydes. The profile for acetaldehyde follows a similar trend to the decline in PFBHA reagent, which demonstrates that even though there is a significant amount of PFBHA remaining the reaction rate can still be affected. The greater robustness of the C_5 and C_6 aldehyde derivatisation to high propanal may in some way relate to the differing affinities of the aldehydes for the PDMS–DVB coating itself. Previous experiments have shown that the PDMS–DVB fibre is significantly less sensitive to underivatised aldehydes with a chain length of less than five [33].

The tolerance of the method to elevated levels of formaldehyde is very useful, since in many matrices this will be present in a significantly larger concentration than the heavier aldehydes. For samples containing elevated levels of other aldehydes, particularly those with a chain length between 2 and 5, inaccuracies in the results are likely to occur if the concentration exceeds the method linear range. To overcome this difficulty a shorter extraction time could be used.

3.3. Method parameters and reagent comparison

Table 2 shows the detection limits, linear range and reproducibility data for the two reagents. Decanal whilst detected using the technique gave very high R.S.D. values and was not considered further. This behaviour is probably caused by the decreasing volatility of the aldehydes with increasing carbon number. Detection limits for PFPH were calculated as the concentration giving a peak height three times the signal-to-noise ratio, whilst for PFBHA peak giving a height twice the average background level was used.

Overall, PFBHA proved to be superior to PFPH for in-fibre derivatisation of aldehydes. It was more sensitive, had less formaldehyde contamination and generally gave a better reproducibility. The lower sensitivity of PFPH was largely the result of a major increase in baseline noise that occurred after

Table 2
Reproducibility, linear range and detection limit data for the developed PFPH and PFBHA in-fibre derivatisation methods

	PFPH				PFBHA			
	Reproducibility (%)[a]	LOD (μg/L)	Linear range (μg/L)	R^2	Reproducibility (%)[a]	LOD (μg/L)	Linear range (μg/L)	R^2
Formaldehyde	10.7	65	65–250	0.9910	10.5	25	25–250	0.9955
Acetaldehyde	4.8	5	5–250	0.9920	5.7	0.5	1–250	0.9999
Propanal	7.5	5	5–1000	0.9986	2.9	0.3	1–250	0.9996
Butanal	4.2	2	2–1000	0.9998	2.2	0.4	1–500	0.9913
Pentanal	3.3	2	2–500	0.9987	1.8	0.1	1–500	0.9987
2-Methylpentanal	2.9	2	2–500	0.9980	1.8	0.5	1–500	0.9973
Hexanal	5.0	1	1–1000	0.9913	3.0	0.5	1–1000	0.9929
Heptanal	5.7	1	1–1000	0.9967	2.5	0.4	1–1000	0.9998
Octanal	10.2	2	2–1000	0.9916	4.8	2	2–1000	0.9995
Nonanal	6.4	2	2–1000	0.9453	10.1	2	2–1000	0.9994

[a] 100 μg/L Aldehyde solution used for reproducibility study (n = 10).

the retention time of the unreacted PFPH, indicating that the high concentration of this compound destabilised or caused some transient contamination of the detector. Reducing the concentration of the PFPH solution to 1 mM did not prevent this effect from occurring. The use of ECD or MS detection may offer a solution to this problem. From these results, it was decided to use the PFBHA method in all the applications tested.

With the exception of formaldehyde, the PFBHA method showed sensitivity of the same order for all the aldehydes investigated up to nonanal as previously reported with EPA method 556, using liquid–liquid extraction [14]. However, the method was less sensitive than previous work in SPME with PFBHA derivatisation with MS [25,26] or ECD [2,7] detection. However, detection limits could be reduced further by finding means of reducing background contamination, longer extraction times and by using one of the more sensitive detectors discussed in previous communications. For the sample matrices investigated in this study, the sensitivity was more than adequate using FID.

Reproducibility of the PFBHA method for most species was similar to those obtained by Vesely et al. [26] for various C_4–C_6 aldehydes in beer using a similar automated technique.

3.4. Applications

The developed method was applied to three different sample matrices; particleboard, white wine and fish. A typical chromatogram for the particleboard is given in Fig. 4, whilst results for the three samples and the respective relative recoveries are given in Table 3. For the wood sample it was no surprise to find the predominant aldehyde was formaldehyde, through its use as an adhesive for particleboard. This could not be quantified as the concentration was significantly above the linear range of the method. All other straight chain aldehydes from C_2–C_9 were also detected in this sample. The remaining aldehydes observed are not added through production of the particleboard and it has been suggested that these are degradation products of part of the wood or secondary metabolites [34]. Hexanal was the second most prevalent aldehyde in this matrix which is in agreement with a previous study that looked at a variety of particleboards and found that emissions of hexanal were significantly higher than those of the other aldehydes investigated (C_5–C_9) [34]. The wine sample contained 9 mg/L acetaldehyde, which is slightly below the typical range of values typically observed for this compound in white wine of between 11 and 493 mg/L [35]. No other aldehydes were observed in this sample, although this may be the result of the high dilution factor required. The raw fish sample contained all of the C_1–C_9 straight chain aldehydes. Formaldehyde concentration was again too high for quantification. Out of the remainder acetaldehyde, propanal and hexanal were the next three most prevalent in this

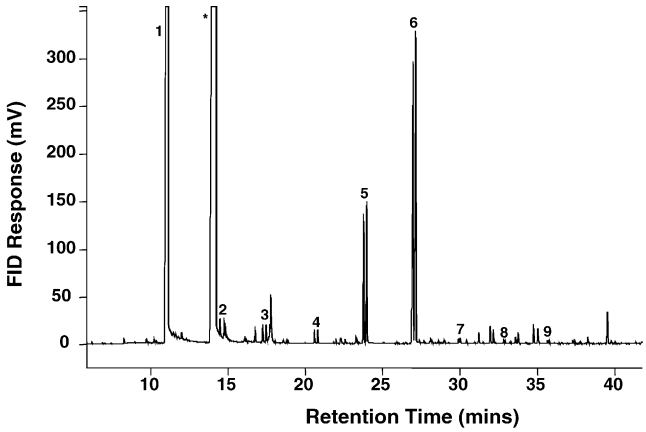

Fig. 4. Chromatogram of 0.02 g of particleboard shavings in 2.0 mL of saturated sodium chloride solution using the PFBHA method. Peaks are as follows (aldehyde peaks refer to PFBHA derivatives): (*) PFBHA; (1) formaldehyde; (2) acetaldehyde; (3) propanal; (4) butanal; (5) pentanal; (6) hexanal; (7) heptanal; (8) octanal; (9) nonanal. The remaining peaks were predominantly unidentified components of the wood sample. For experimental conditions see text.

Table 3
Observed concentrations and relative recoveries of aldehydes observed in particleboard, white wine and raw fish samples using the PFBHA method

	Particleboard		Raw fish meat		Dry white wine	
	Concentration, μg/g (R.S.D., %)	Relative recovery, % (R.S.D.,%)[a]	Concentration, ng/g (R.S.D., %)	Relative recovery, % (R.S.D., %)[b]	Concentration, mg/L (R.S.D., %)	Relative recovery, % (R.S.D., %)[c]
Formaldehyde	>100	–	>2000	–	nd[d]	–
Acetaldehyde	3 (7)	94 (8)	102 (5)	92 (2)	9 (8)	91 (7)
Propanal	2 (5)	95 (2)	96 (9)	109 (6)	nd	–
Butanal	1 (6)	97(4)	7 (9)	83 (8)	nd	–
Pentanal	10 (14)	91 (4)	4 (9)	100 (11)	nd	–
Hexanal	53 (15)	91 (15)	39 (13)	95 (4)	nd	–
Heptanal	1 (8)	107 (1)	4 (6)	81 (11)	nd	–
Octanal	2 (5)	72 (3)	3 (13)	91 (8)	nd	–
Nonanal	2 (2)	38 (6)	21 (5)	93 (13)	nd	–

[a] Spiked amount for the wood sample was 10 ng for C_2, C_7, C_8, C_9, 20 ng for C_3, C_4 and 200 ng for C_5, C_6.
[b] Spiked amount of aldehydes for the fish was 20 ng for all aldehydes tested.
[c] Spiked amount of acetaldehyde in wine was 100 ng.
[d] Not detected.

sample, which are thought to occur from the lipid oxidation of omega-3 and omega-6 fatty acids [36,37]. With the exception of formaldehyde, the results were generally lower than the levels observed in a previous study of several different types of fish, although this would be expected since in that study the fish were cooked at 200 °C during sampling, which would accelerate formation of oxidation products [37]. Relative recovery was good to excellent in all the samples investigated, except for nonanal in the wood sample.

4. Conclusions

This detailed investigation of aldehyde headspace analysis, using SPME–GC–FID with in-fibre derivatisation showed that PFBHA was a superior in-fibre derivatisation reagent to PFPH under the investigated conditions, with detection limits at the low to sub microgram per litre level. The automated method can be successfully applied to a variety of sample types. Studies of the reaction kinetics demonstrated that with both reagents production was diffusion limited, with the 'bottleneck' most likely occurring at the sample/headspace interface. Therefore, both extraction temperature and agitation conditions needed to be optimized during method development. The method was able to handle samples containing elevated levels (10 000 μg/L) of formaldehyde, whilst the presence of elevated levels of other aldehydes, such as propanal will require the use of shorter extraction times in order to maintain the accuracy of the method. Further development of the technology will include design coatings containing immobilized derivatisation reagents, which will only dissociate under desorption conditions.

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