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# Review

# O-(2,3,4,5,6-Pentafluorophenyl)methylhydroxylamine hydrochloride: a versatile reagent for the determination of carbonyl-containing compounds

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ABSTRACT

A review on the use of O-(2,3,4,5,6-pentafluorophenyl)methylhydroxylamine hydrochloride (PFBHA) for the determination of carbonyl-containing compounds is presented. PFBHA has been used in the determination of such diverse compounds as thromboxane $B_2$, prostaglandins, amygdalin and a variety of other aldehydes, ketones and acids. PFBHA has been used for the determination of these compounds found in water, blood, urine, air and even clothing. The review covers literature referenced in *Chemical Abstracts* from 1975, when PFBHA was first synthesized, through March 1992.

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## 1. INTRODUCTION

O-Substituted derivatives of hydroxylamine are useful reagents for the determination of carbonyl-containing compounds. Of particular importance is the fluorine-containing O-substituted hydroxylamine O-(2,3,4,5,6-pentafluorophenyl)methylhydroxylamine hydrochloride (Fig. 1). This compound has appeared in the literature by the name of O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride or pentafluorobenzyloxylamine and has been abbreviated as PFBHA, PFBOA and PFBHOX. PFBHA (CAS Registry No. 57981-02-9), as it is referred to in this review, has been used as a derivatizing agent in a wide variety of biomedical and environmental investigations.

PFBHA, like hydroxylamine, reacts with carbonyl functional groups to produce the corresponding oximes (Fig. 2). Oxime formation is pH dependent and proceeds readily in both aqueous and organic solutions. The oximes are easily extracted into organic solvents, with unreacted PFBHA being removed through acidic washings. The oximes can be separated by gas chromatography (GC) and analyzed with a variety of detectors. Electron-capture detection (ECD) is particularly sensitive for the determination of these pentafluoro oxime derivatives.

PFBHA has been used in the determination of such diverse compounds as thromboxane B2, prostaglandins, amygdalin and formaldehyde. It has also been used in the determination of aldehydes, ketones and acids found in water, blood, urine, air and even clothing. This review will discuss the methodologies for the use of PFBHA in the determination of a variety of compounds using gas chromatography–mass spectrometry (GC–MS), GC-flame ionization detection (FID) or GC–ECD. This review covers literature referenced in *Chemical Abstracts* from 1975, when PFBHA was first synthesized, through March 1992.

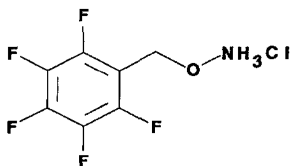
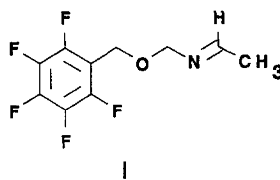



Fig. 1. O-(2,3,4,5,6-Pentafluorophenyl)methylhydroxylamine hydrochloride (PFBHA).



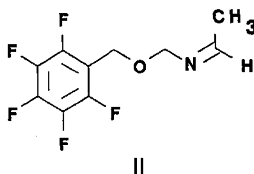




Fig. 2. (I) *E* and (II) *Z* geometrical isomers of acetaldehyde O-[(pentafluorophenyl)methyl] oxime.

## 2. NOMENCLATURE OF PFBHA DERIVATIVES OF CARBONYL-CONTAINING COMPOUNDS

As Fig. 2 indicates, the presence of three different groups around the carbon-nitrogen double bond produces geometrical isomers. The *E*/*Z* method of nomenclature will be used to describe specific geometrical isomers. In this system, functional groups about the double bond are prioritized [1], with the *Z* isomer containing the highest priority groups on the same side of the double bond. The *E* isomer contains the two highest priority groups on opposite sides of the double bond.

## 3. SYNTHESIS AND INITIAL USE OF PFBHA

The synthesis and use of PFBHA was first described in a series of papers by three groups of researchers in 1975 and early 1976. In the first paper, Koshy *et al.* [2] at Upjohn used PFBHA to form monooximes of 3,17- and 20-keto steroids and dioximes of 3,17- and 3,20-diketo steroids. Although their experimental section indicated that PFBHA was synthesized at the Upjohn Research Laboratories and acknowledged Dr. G. A. Youngdale for the synthesis, the actual synthetic method was not described. In 1975, Nambara *et al.* [3] also described both the synthesis and use of PFBHA for the analysis of 17-keto steroids.

Youngdale's synthetic method was published in

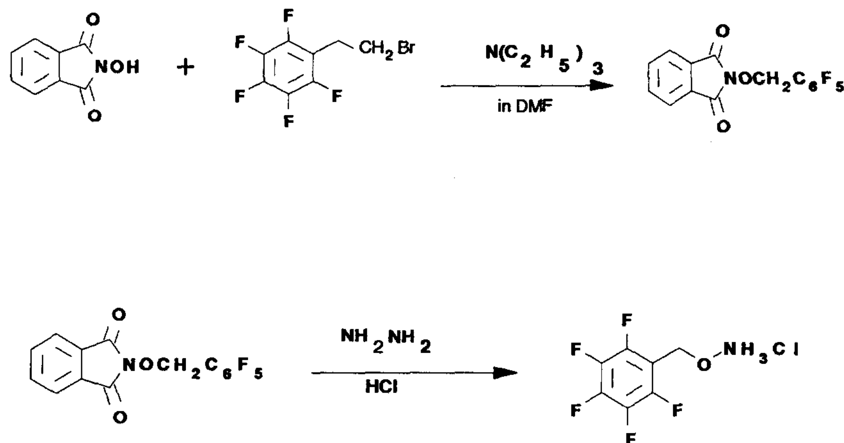



Fig. 3. Youngdale's [4] synthetic method for PFBHA.

1976 [4], although it had been accepted for publication in early 1975. The method, described as a modification of methods developed by McKay *et al.* [5] for the synthesis of O-substituted oxyamines, is shown in Fig. 3. Except for a few differences, such as molar amounts used and choice of solvents, the method described by Nambara *et al.* is essentially the same. None of the investigators included spectroscopic data in support of the structure or purity of PFBHA or the oximes.

The general approach for the determination of steroids by both the Koshy and Nambara groups was to react the specific steroid in a solution of pyridine containing excess of PFBHA at elevated temperatures (60–65°C) for either 30 or 60 min. Time and temperature conditions or PFBHA and steroid concentrations were not optimized. The Koshy group removed the pyridine under nitrogen and redissolved the oxime in cyclohexane. This solution was then washed once with distilled water, dried with sodium sulfate and analyzed by GC–FID, GC–ECD or GC–MS. Further experiments were performed using thin-layer chromatography (TLC). The hydroxy functional groups were not derivatized. The method was used only for standards in pyridine and was not extended to detect steroids in biological matrices.

After initial oxime formation in the PFBHA pyridine solution, the Nambara group used 0.1 *M* HCl, 0.1 *M* NaOH and water washings followed by treatment with hexamethyldisilane and trimethylchlorosilane. Pyridine was removed and the samples redissolved in hexane for analysis by GC–ECD or GC–FID. Individual samples in pyridine were prepared in this manner. The method was then used to determine levels of dehydroepiandrosterone added to human plasma.

A number of interesting and significant conclusions were reached in these initial papers, perhaps the most important being the demonstration of the existence of geometrical isomers by the Koshy group. Using TLC and longer GC columns, both geometrical isomers of testosterone and 7α,17α-dimethyl-19-nortestosterone were isolated and detected after reaction with PFBHA. Except for this demonstration, however, the optimized analytical conditions necessary to separate any of the other possible steroid oxime *E*/*Z* isomers were not described. GC determination of the various steroid oxime derivatives used the unresolved isomers. As little as 5 pg of the unresolved testosterone oxime were detected with GC–ECD. Detected amounts of other steroids ranged from 50 to 200 pg, with detector response ranging from $1.54 \cdot 10^4$ C/mol for andosterone to $9.60 \cdot 10^4$ C/mol for progesterone.

Koshy *et al.* further demonstrated the stability of the oxime derivatives in milligram or microgram amounts by determining recovered amounts after solvent partitioning (GC–FID) and TLC separation ($^3$H-labeled scintillation spectrometry), again using testosterone and 7α,17α-dimethyl-19-nortestosterone. Recovery was quantitative for solvent

partitioning and was between 85 and 97% by TLC.

Nambara *et al.* focused primarily on the 17-keto steroids because the 11-keto steroids failed to react with PFBHA under the described experimental conditions. Through a series of spiking experiments, they found that 84.5% of the spiked dehydroepiandrosterone in human plasma could be recovered as the oxime. It was further suggested that PFBHA was added to biological material to remove ketones in these matrices.

These papers were important for demonstrating that PFBHA could be used for the determination of steroids through formation of their oximes. The oximes were found to be easily separated and detected by GC–ECD and were stable under a variety of analytical conditions. This early work had its limitations; none of the groups synthesized or characterized pure oximes to be used as analytical standards, optimized the reaction conditions, or measured steroid levels in actual biological samples. Nonetheless, these investigators presented an important new derivatizing agent and demonstrated that PFBHA had the potential to be an extremely useful analytical agent.

## 4. BIOLOGICAL APPLICATIONS OF PFBHA

### *4.1. Prostaglandins and thromboxanes*

The need of researchers to detect submicrogram levels of prostaglandins and thromboxane $B_2$ in biological matrices soon provided another use for PFBHA. These methods are listed in Table 1. In 1977, Fitzpatrick *et al.* [6] demonstrated that PFBHA could be used to form the pentafluorobenzyl oxime (PFB oxime) of prostaglandins (PGs) and thromboxane $B_2$ (TX).

PG and TX were isolated from washed platelets and processed through a three-step derivatization which included methyl esterification and oxime formation. Samples were analyzed using either GC–ECD or GC–MS.

Although no analytical data were supplied, TX was reported to be recovered nearly quantitatively (90%) from washed platelets. The TX methyl ester PFB oxime was then formed and extracted quantitatively with two hexane washings. A number of PGs ($PGA_2$, $PGB_2$, $PGE_2$ and $PGD_2$) were resolved and were clearly separable from the geometrical isomers of TX, although those of PGs could

TABLE 1

SUMMARY OF PFBHA METHODS FOR THE DETERMINATION OF PROSTAGLANDINS AND THROMBOXANES

| Compound type | Matrix type | Detection method | Chromatographic method | Ref. |
|---|---|---|---|---|
| Mono- and diketo steroids | Pyridine | ECD, FID, MS | Packed column GC, TLC | 2 |
| Mono- and diketo steroids | Pyridine, plasma | ECD, FID | Packed column GC | 3 |
| Prostaglandins, Thromboxane | Platelets | ECD, MS | Packed column GC | 6 |
| Prostaglandins, Thromboxane | Platelets | UV(254 nm), ECD | $C_{18}$ liquid chromatography, packed column GC | 7 |
| Prostaglandins, Thromboxane | Cell preparations (Balb 3T3, Balb 3T12) | ECD | Capillary GC | 8 |
| Prostaglandins, Thromboxane | Mouse peritoneal macrophage cells | MS | Capillary GC | 9,10 |
| Prostaglandins | Blood | ECD | Capillary GC | 11 |
| Prostaglandins, Thromboxane | Semen, rat aorta, dog serum, trout gill | ECD | Capillary GC | 12 |
| Prostaglandins | Phosphate buffer, fetal calf serum, lung fibroblasts, plasma | ECD | Capillary GC | 13 |
| Prostaglandins | Pyridine | NCI-MS | Capillary GC | 14 |

not be separated. Peak-height ratios of the *E*/*Z* isomers of TX varied by only ±5%, providing a rough qualitative assurance measure; any deviation beyond this was claimed to result from co-eluting and interfering peaks.

Derivatization reproducibility experiments for the TX methyl ester PFB oxime at the 50–375-ng level ranged from ±11 to ±42%. This poor reproducibility was attributed to both the labile nature of TX and to difficulties associated with carrying out three successive derivatizations on nanogram amounts of material.

In a subsequent paper, the Fitzpatrick group [7] expanded upon its earlier work by demonstrating that PGs and TX could be determined with liquid chromatographic (LC) separation using a 254-nm fixed-wavelength detector. Modifications in the derivatization procedure included formation of the PFB oximes of PG PFB esters and methyl esters. The oximes of the PFB esters were detected at between 60 ng (15-methyl-$PGB_2$) and 930 ng ($PGE_2$). The oximes of the methyl esters could not be adequately detected. In parallel experiments, the *p*-nitrobenzyl oximes of both the methyl and PFB esters were detected at significantly lower concentrations than the PFB oxime. LC separation of the PGs allowed resolution of geometrical isomers in some instances.

Derivatization reproducibility, as determined by GC–ECD, did not improve from that reported in earlier work for either the TX or PG derivatives [6]. The methyl ester PFB oximes were more sensitive to detection by GC–ECD than the PFB ester–PFB oximes of the prostaglandins. Detection limits were between 15 and 300 pg for the methyl ester PFB oximes and between 30 and 400 pg for the PFB ester–PFB oximes. The *E*/*Z* isomers of the methyl ester oximes were chromatographically separated.

Better separation of prostaglandins was achieved by Fitzpatrick *et al.* [8] through the use of glass capillary GC–ECD. Samples were methyl esterified as described earlier [7] and, after treatment with pyridine containing of excess PFBHA, were washed with water at pH 3.0. Prostaglandin and thromboxane methyl ester oximes were then converted into trimethysilyl ethers. A 1-$\mu$l volume of the final derivative was used for analysis by GC–ECD. The detector was equipped with a tee to supply a make-up gas flow, which was found to be optimum at about 15 ml/min [helium–argon (90:10)]. The detector response decreased logarithmically with increasing make-up flow-rate and a fixed column flow-rate (helium) of 22.5 ml/min. Derivative separation and detection equaled or exceeded that found for packed column GC–ECD. The methyl ester PFB oxime trimethysilyl ethers of $PGD_2$, $PGE_2$ $TXB_2$ and 6-keto-$PGF_{1\alpha}$ were not completely resolved, although the *E*/*Z* isomers of the oximes were distinguished. Using this method, 100 pg of $PGH_2$ were detected at a signal-to-noise ratio of 20:1. These methods were used to monitor metabolic profiles of $PGH_2$ in cell preparations.

Rosello and co-workers [9,10] demonstrated the use of capillary GC–MS for the determination of the major metabolites of the cyclooxygenase pathway of arachidonic acid from mouse peritoneal macrophage cells using methyl ester–PFB oxime–butylboronate–TMS derivatives of $TXB_2$ and 6-keto-$PGF_{1\alpha}$. This involved a four-step derivatization,

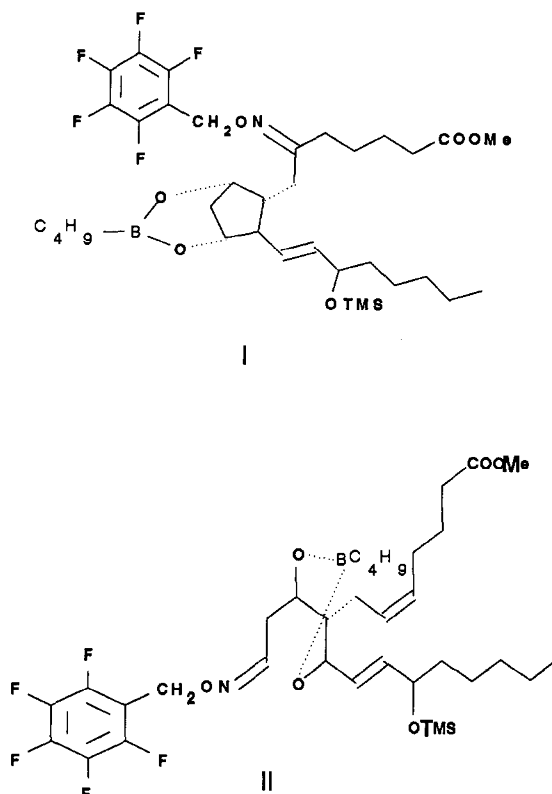



Fig. 4. PFBO–NBB–TMS–Me derivatives of (I) 6-keto-$PGF_{1\alpha}$ and (II) $TXB_2$.

beginning with esterification followed by oximation with PFBHA, boronation and silylation. Fig. 4 shows the resulting PFBO–NBB–TMS–Me derivatives of the 6-keto-$PGF_{1\alpha}$ and $TXB_2$. Samples were chromatographed using glass capillary columns.

The *E*/*Z* isomers were easily resolved, as were various PGs and TX mixtures. The PFB–NBB–TMS–Me derivatives generally showed better chromatographic separation than the PFB–TMS–Me derivatives. The *E*/*Z* isomers of 6-keto-$PGF_{1\alpha}$ co-eluted under these conditions, although they could be separated using less polar OV-1 or OV-101 stationary phases. A characteristic loss of M − 197 (attributed to loss of $C_6F_5CH_2O$) was noted in the mass spectra of the derivatives of 6-keto-$PGF_{1\alpha}$ and $TXB_2$.

In a lengthy procedure, Christ-Hazelhof and Nugteren [11] used the PFB derivative of 6-oxo-$PGF_{1\alpha}$, a hydrolysis product of prostacyclin, to determine prostacyclin ($PGI_2$) levels in blood.

Recoveries using this multi-step process were reported to be between 57 and 60%. No prostacyclin was detected in normal human venous peripheral whole blood or blood plasma or in samples taken during and after hyperventilation, indicating that prostacyclin is not a normally circulating hormone. Ten micrograms of sodium prostacyclinate in 2.5 ml of saline infused into one arm vein of a male volunteer for 5 min were detected in whole blood samples drawn from the other arm at 2, 3, 4, 5, 7, 9, and 11 min. Results from this experiment produced levels of 400 pg of prostacyclin per milliliter of whole blood. This showed the half life of infused prostacyclin to be between 2 and 4 min.

Optimized GC–ECD conditions for the analysis of PG and TX as their PFB–TMS derivatives were described by Mai *et al.* [12]. Using the method of Fitzpatrick *et al.* [7], PGs and TX were esterified, oximated and silylated. Samples were detected by $^{63}$NiECD or GC–MS using selective ion monitoring (SIM) (no ions were specified). Glass columns were either 2.8 m × 0.32 cm I.D. or 4.7 m × 0.32 cm I.D. The suitabilities of ten separate stationary phases were tested, with 3% SE-30, 3% OV-1, 3% SP-2100 and 3% SP2100 providing the best general separations for PG pentafluorobenzyl derivatives. Capillary columns were necessary to achieve the best separations.

Column temperatures were optimized using a 2.8 m × 0.32 cm I.D. 3% OV-101 glass column and the $PGE_2$–PFBO–PFBE–TMS derivative. Optimum chromatographic resolution was achieved by holding the column at 270°C for 10 min, then programming to 285°C at 5°C/min. It appears that no effort was made to optimize the injector temperature, which was reported as 270°C. The detector response was found to increase with increasing detector temperature, with the highest sensitivity achieved at 350°C. Argon–methane (90:10) carrier gas at a flow-rate of 16 ml/min offered the best detector response. As a general rule, decreases in response were observed with increasing flow-rates, and poor separations were achieved at low flow-rates. Plotting ECD response against the amounts of PG used for derivatization provided calibration graphs which showed the same overall trends as those observed by Fitzpatrick *et al.* [8] using glass capillary column GC–ECD. Relative response factors on two separate columns of fourteen PGs using 1a,1b-dibromo-$PGF_{1\alpha}$ as internal standard are reported. The ratios of the *E*/*Z* isomers of the nine PFB oxime PFB ester TMS ether derivatives were reported as the ratio of the peak area of the first peak to the second peak. No attempt was made to assign configurations to specific peaks or to observe the effects of various operating conditions on the ratios.

Rosenfeld *et al.* [13] demonstrated a method for the solid-phase sample preparation for PGs using PFBHA as an oximation agent. XAD-2, a styrene–divinylbenzene cross-linked polymeric macroreticular resin, was used as the sorbent for $PGE_2$ from various biological samples (phosphate buffer, biological incubate–10% fetal calf serum and human lung fibroblasts and human plasma).

TLC using silica gel plates and a toluene–ethyl acetate–methanol (55:45:2.5, v/v/v) of $^{14}$C-labeled and authentic $PGE_2$ derivatives was used to obtain reaction yields. The yields were calculated to be 73 ± 4% (buffer) and 54 ± 9% (plasma). Total radiolabel recovery was 85% from all matrices. Incomplete reaction products such as oximation, esterification or unreacted $PGE_2$ accounted for the remaining detected products and were attributed to matrix affects. It was questioned whether proteins containing sulfhydryl groups caused the lowered recovery. Reaction of $PGE_2$ in an aqueous solution containing physiological concentrations of added

serum albumin gave recoveries (63 ± 5%) similar to those for buffered solutions, however, indicating other causes. The $E/Z$ isomers could be separated using TLC and GC. The $Z$ isomer was assigned to the minor GC peak, although no supporting spectral evidence was provided to confirm this assignment. Linear calibration graphs from 25 to 250 ng/ml were obtained using a 1–2-$\mu$l volume of the derivatized sample with GC–ECD and on-column injection.

In summary, a significant amount of research has been focused on the isolation and detection of PGs and TXs from biological matrices using PFBHA. Usually, multi-step derivatizations are required to prepare these compounds for analysis. PFBHA has been used for reaction of the carbonyl functional groups of these compounds to form the resulting oxime. Generally, $E/Z$ isomers of these compounds are formed and can be separated by TLC, HPLC and GC. Recovery and reproducibility from the multi-step derivatizations varied in the different studies, ranging from 63% to 90%. The detection limits were reported to be in the range 25–50 ng and the derivatization reproducibility was 42%.

GC–ECD has been shown to be the most sensitive method for the determination of the PFB oxime derivatives. SIM-MS can approach the sensitivity of ECD, although a significant amount of work remains to be done on determination of the actual detection limits. The addition of two electron-capturing groups, the PFB oxime and the PFB ester, did not lower detection limits. Generally, these double derivatives had limits of detection much poorer than those for the single derivatives. This trend was also observed in a study by Waddell *et al.* [14] using GC–negative-ion chemical ionization mass spectrometry (GC–NICI-MS). The fragmentation patterns of various PFB oxime derivatives of 6-oxo-$PGF_{1\alpha}$ were studied using methane as the reagent gas, an ionizer temperature of 200°C and an ion source pressure of 27 Pa. Two fragment ions, of $m/z$ 178 $[C_7F_4H_2O]^-$ and $m/z$ 196 $[C_7F_5HO]^-$, were found to carry most of the total ion current for the PFB oxime, methyl ester, tris-TMS derivative of 6-oxo-$PGF_{1\alpha}$. The limit of detection for the $m/z$ 686 ion $[M{-}C_3H_{10}SiOF]^-$ was reported to be 800 pg on-column.

### 4.2. *Determination of other metabolic products and drugs*

PFBHA has been successfully used for the determination of a number of drugs and metabolic products in plasma, urine, feces and tissue (Table 2). Park *et al.* [15] detected arildone (Fig. 5), an anti-

TABLE 2

SUMMARY OF PFBHA METHODS FOR THE DETERMINATION OF METABOLIC PRODUCTS AND DRUGS

| Compound type | Matrix type | Detection method | Chromatographic method | Ref. |
|---|---|---|---|---|
| Arildone | Urine, plasma | ECD | Capillary GC | 15 |
| Geranylgeranylacetone | Serum | MS | Packed column GC | 16 |
| Acetone, Acetoacetic acid | Urine | FID | Packed column GC | 17 |
| 4-Hydroxynonenal | Rat heart, liver, adrenal, testis | NCI-MS | Capillary GC | 18 |
| Monosaccharides | Pyridine | FID, MS | Packed column GC | 19 |
| Aldehydes, ketones, oxo acids | Water, urine, plasma, amniotic fluid | FID, MS | Capillary GC | 20 |
| Acetaldehyde | Blood | ECD | Packed column GC | 21 |
| Aldehydes, ketones, oxo acids | Urine, plasma, amniotic fluid | MS | Capillary GC | 22 |
| Aldehydes, ketones, oxo acids | Urine, plasma, amniotic fluid | MS | Capillary GC | 23 |
| *trans*-4-Hydroxy-2-nonenal (HNE) | Platelets, monocytes, plasma, oxidized low-density lipoprotein | NCI-MS | Capillary GC | 24 |
| 2,5-hexanedione | Urine, serum | FID, ECD | Capillary GC | 25 |
| Indole-3-pyruvate | Tomato shoots | ECD, MS | Capillary GC | 26 |

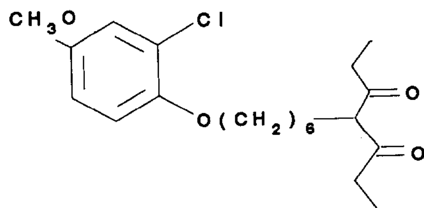



Fig. 5. Arildone, 4-[6-(2-chloro-4-methoxyphenoxy)hexyl]-3,5-heptanedione).

viral agent, in urine and plasma using packed column GC–ECD. Linear dynamic ranges of 10–120 ng/ml for plasma and 2.5–20 ng/ml in urine were achieved. The minimum quantifiable level of the oxime derivative of arildone was 1.4 ± 0.2 ng/ml in urine and 6.4 ± 0.1 ng/ml in plasma. The precision was 5.5% in urine and 6.4% in plasma.

A number of problems were reported in the derivatization of arildone, a diketone. Generally, mixed *E*/*Z* isomers could not be resolved using the short packed column and all quantification was done on the co-eluting isomer peaks. Further, the column required conditioning by injection of high concentrations of derivatives prior to injection of samples.

The anti-ulcer drug geranylgeranylacetone (GGA) [16] was determined in human serum using GC–MS. Generally, 1 ml of serum was used and a multi-step clean-up was required before oxime derivatization could be undertaken.

GC–MS of the derivatives showed four peaks attributed to the formation of *E*/*Z* isomers from each of the *cis*/*trans* isomers (*cis*/*trans* about internal double bonds) of GGA. Although no other spectral evidence was provided, the *E* isomer of the *cis*-GGA starting isomer was assigned by its spectrum showing the more intense *m*/*z* 320 ion (eight times that in the spectrum for the *Z* isomer). The formation ratio of isomers was almost constant, which provided an opportunity to use SIM for quantification of the *m*/*z* 320 ion of the *E* isomer. The linear dynamic range was 1–1000 ng/ml, with a variation of less than 5%. Experiments with different concentrations of PFBHA showed that the optimum formation of the GGA oxime was achieved using 5 mg of PFBHA per 0.2 ml pyridine.

Kobayashi *et al.* [17] demonstrated a GC method for the determination of acetone and acetoacetic acid in urine. It was reported that acetoacetic acid underwent rapid decomposition in acidic media to yield acetone. Linear calibration graphs in the range 17–862 μmol/l for acetone and 20–980 μmol/l for acetoacetic acid were obtained for urine samples spiked with the analytes and diluted ten-fold. Reproducibilities for identical sample solutions containing 345 μmol/l of acetone and 490 μmol/l of acetoacetic acid were 1.7% and 2.0% ($n$ = 5), respectively. The absolute sensitivity for acetone was 3.45 μmol/l for acetone and 10 μmol/l for acetoacetic acid.

4-Hydroxynonenal was detected at the 10–100-pg level from the heart, liver, adrenal and testis of rats using NCI-MS [18]. The reaction was carried out in the presence of a buffer (pH 7.0); no evidence was provided, however, to demonstrate that this was the optimum pH under which oximes could be formed and extracted. Samples were analyzed using GC–NICI-MS. Ammonia was the reagent gas and NICI mass spectra were obtained by SIM using the ion of *m*/*z* 152. A comparison between isomer intensity ratios of oximes extracted from rat livers and those from authentic standards showed that the two were equivalent. NICI-SIM-MS was between 20 and 100 times more sensitive than electron impact (EI)-MS; the most abundant ion in this method was *m*/*z* 200.

The optimum conditions necessary for the determination of the PFB oxime–acetyl ester derivatives of neutral monosaccharides were studied by Biondi *et al.* [19]. Conditions of solvent type, reaction time and temperature were examined, but other factors, such as pH, extraction number, PFBHA concentration and chromatographic conditions, were not investigated. Reaction of PFBHA in pyridine for 20 min at 80°C was found to provide the optimum conditions for oxime formation. Incomplete derivatization occurred when other solvent combinations were used, as demonstrated by the appearance of extra peaks in the chromatograms. No information was provided on experiments to optimize the chromatographic conditions, although the optimized conditions were described (180°C for 4 min, then increased at 5°C/min to 240°C and held for 10 min, injector temperature 270°C and detector temperature 240°C).

Recovery studies were performed using milligram amounts of glucose to form the Ac–PFB–Gl derivative. Excess of PFBHA was removed by passing the reaction mixture through a Bond Elut SCX sul-

phonated silica column which was reported to retain PFBHA selectively. The MS of the O-PFB oxime acetates of frucose, arabinose, xylose, glucose, galactose and mannose were provided. Common to all spectra was the base peak at *m/z* 181, which was attributed to the $C_7H_2F_5$ ion. Recoveries were reported to be between 87 and 95% for the 10-nmol range and between 94 and 98% for the 100-nmol range. No decrease in peak heights was observed for derivatives stored at room temperature for 2 days.

Hoffmann and Sweetman [20] reported on the oxime–TMS ester derivatives of approximately twenty physiologically important aldehydes, ketones and oxo acids. Aqueous samples were used for initial method development, which was then generalized for use with urine, plasma and amniotic fluid.

Silicic acid column clean-up of samples to remove unreacted PFBHA and unwanted artifacts was described as an important step in the reaction procedure. It was reported that PFBHA reacted with silylating reagents to produce corresponding PFB–TMS derivatives as byproducts which could be chromatographed. This could be an obvious source of problems if chromatographic retention times overlapped with the analytes of interest. The *E/Z* isomers of many of the analytes were observed as double chromatographic peaks, with the second peak generally much more prominent than the first and increasing with increasing complexity of the R group. Succinylacetone yielded four product peaks which were attributed to all possible *E/Z* combinations resulting from the existence of two oxo groups. Relative standard deviations of between 15 and 78% were observed for samples between 0.20 and 0.01 $\mu$mol and between 2 and 7% for samples between 1.00 and 0.5 $\mu$mol. This would indicate that determinations of levels below 0.02 $\mu$mol could prove difficult using GC–FID.

A method for the determination of acetaldehyde in blood using PFBHA derivatization and GC–ECD was described by Tomita *et al.* [21]. Blood samples spiked with 3 and 9 $\mu M$ acetaldehyde were recovered at 94.7 and 99.8%, respectively. Samples spiked with 9 $\mu M$ acetaldehyde and 20 $\mu M$ ethanol showed a recovery of 96.8%. The detection limit was about 1 $\mu M$. Samples containing ethanol had to be extracted within 2 min, as ethanol formed acetaldehyde under these analytical conditions. A further complication was that samples spiked with both acetaldehyde and ethanol and left at 26°C before work-up showed a significant decline in the amount of acetaldehyde recovered over time, with levels disappearing within 60 min.This was attributed to the binding of acetaldehyde to erythrocytes or plasma proteins and the resulting oxidation of acetaldehyde by aldehyde dehydrogenase. Another problem was noted with the high background levels of formaldehyde present in blank and spiked samples.

This was one of the first reports using lowered temperatures (ice-bath temperatures) instead of elevated temperatures for the formation of the PFB oxime derivative. Despite the obvious problem of acetaldehyde loss, relatively high recoveries were achieved at these low temperatures. One defect of the study was that the effects of temperature on the formation of the acetaldehyde oxime were not fully addressed. ECD temperatures were also generally lower than those reported as being optimum for oxime derivatives.

In a continuation of their earlier work, Hoffmann *et al.* [22] described the batchwise isolation of the oximes of aldehydes, ketones and oxo acids through the use of liquid partition chromatography. This method was used to isolate these derivatives from urine, plasma and amniotic fluid samples. Oxime formation and chromatographic conditions were as described in their earlier work [20]. An interesting variation on the earlier method was the addition of 20 $\mu$l of a 2.5 g/l rosolic acid solution as a pH indicator. After initial reaction with PFBHA at a pH of 3.5, the pH was adjusted to between 7 and 8, as indicated by the rosolic acid color change. After lyophilization, pH adjustment and liquid partition chromatography, the eluate was titrated with 10 mmol/l NaOH in methanol. Titration to the endpoint of red rosolic acid provided a measure of the total organic acid content of each sample. Mass spectrometry of the PFBHA derivatives showed that the pentafluorotropylium ion $[C_6F_5CH_2]^+$ at *m/z* 181 was the most or second-most intense ion generated from all the oxo acids, aldehydes and ketones analyzed. This ion was then used for the quantification of the various oxime derivatives by SIM-MS.

Hoffmann and Sweetman [23] provided EI mass spectral data for a number of oxo acids, aldehydes

and ketones from biological samples. These samples included amniotic fluid, plasma and urine.

A method for the determination of the lipid peroxidation product *trans*-4-hydroxy-2-nonenal (HNE) in platelets, monocytes, plasma and oxidized low-density lipoprotein in the low ng/ml range was developed by Selley *et al.* [24]. Samples were analyzed by GC–NCI-MS using deuterated HNE as the internal standard. HPLC was performed using a $C_{18}$ reversed-phase column to separate *E*/*Z* isomers of the HNE oxime derivative, so that proton nuclear magnetic resonance (NMR) studies of the isomers could be undertaken. Synthetic PFB oximes of HNE were prepared and NMR spectroscopy performed on the separated isomers. Except for the assignment of the formyl proton adjacent to oxime ($\delta$ 7.70 for the *Z* isomer and $\delta$ 7.04 for the *E* isomer), no further NMR data were provided. This method did allow the assignment of the larger and later eluting peak by HPLC as the *E* isomer (Fig. 6). The *E*/*Z* isomers of the PFB oxime TMS ether showed significantly different relative ion abundances by NCI-MS using ammonia as the reagent gas. The *E*/*Z* ratios were calculated to be 1:0.25 for *m*/*z* 303, 1:0.26 for *m*/*z* 373 and 1:3.32 for *m*/*z* 333, indicating important MS differences between the geometrical isomers.

Kezic and Monster [25] published a note on the determination of 2,5-hexanedione (25HD), a metabolite of *n*-hexane, in urine and serum by GC–FID and GC–ECD. Three isomer peaks were observed for the oxime of 25HD. Detection limits were reported to be 4 $\mu$g/l with ECD and 50 $\mu$g/l with FID. The precision was 3.8% using ECD and 4.5% with FID, with linearity ranges of 4–100 $\mu$g/l for ECD and 0.05–10 mg/l for FID. 25HD could be detected in the serum and urine of individuals exposed to 180 mg/m$^3$ of *n*-hexane. No quantitative results were reported, although blank urine contained 6.4 $\pm$ 2.6 $\mu$g/l of background 25HD.

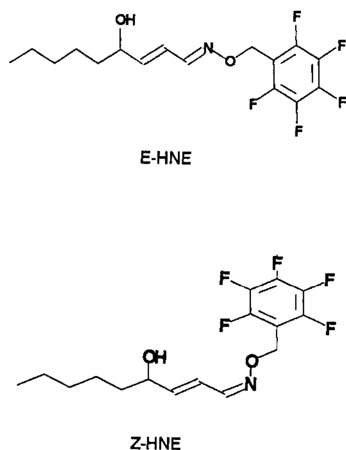



Fig. 6. *E* and *Z* isomers of the PFBHA oxime derivative of *trans*-4-hydroxy-2-nonenal (HNE).

In a study to determine if indole-3-pyruvate (IPyA) is an intermediate in the biosynthesis of indole-3-acetic acid from tryptophan in tomato shoots, a method for the measurement and detection of IPyA using PFBHA derivatization and MS detection was developed [26]. Analysis was performed using GC–ECD or GC–MS. PFB–IPyA methyl ester could be detected by GC–ECD at a level of 50 pg from calibration graphs and at levels between 8.4 and 10.3 ng gf. wt. from plant tissue as compared with tritiated standards. No information was provided on the possible formation of isomers.

PFBHA can be used for a wide range of biological applications. The solubility of PFBHA in various biological fluids and its reactivity over a wide pH range make it a highly desirable derivatizing agent. Excess of reagent can be removed through column chromatography or acid washing. Many of the aldehydes studied formed *E*/*Z* isomers which posed varying levels of difficulty relative to resolution and quantification. Most researchers stated that the ratio of the isomers was stable, but few groups provided sufficient data to support this claim.

### *4.3. PFBHA as a tool for indirect measurements: enzymatic reactions*

A number of researchers have exploited the ease with which PFBHA can be used in aqueous solutions to monitor enzymatic reactions (Table 3). PFBHA was used to derivatize specific carbonyl compounds produced as products from these reactions. In 1981, Kawai *et al.* [27] showed that PFBHA could be used to monitor amygdalin concentrations in aqueous solutions by determination of benzaldehyde formed as a result of the enzymatic hydrolysis of amygdalin by $\beta$-glucosidase. Determination of the derivatives was carried out using either packed or capillary column GC–FID or GC–ECD. Solutions were buffered at pH 4.8 to achieve

TABLE 3

SUMMARY OF PFBHA METHODS FOR THE ANALYSIS OF ENZYMATIC REACTIONS

| Compound type | Matrix type | Detection method | Chromatographic method | Ref. |
|---|---|---|---|---|
| Benzaldehyde | Aqueous solutions | ECD, FID | Packed column GC | 27 |
| Formaldehyde, Methoxyphenoxy-acetaldehyde | Aqueous solutions | FID, MS | Packed column GC | 28 |
| Formaldehyde | Aqueous solution | FID, ECD | Packed column GC | 29 |
| Formaldehyde | Aqueous solution | FID | Packed column GC | 30 |
| Formaldehyde | Catalaze–hydrogen peroxide aqueous solution | ECD | Packed column GC | 31 |

both optimum β-glucosidase activity and oxime formation. No experimental results were described showing how this value was achieved. Separation of *E*/*Z* isomers was not achieved using the chromatographic conditions described.

PFBHA oximes of formaldehyde and methoxyphenoxyacetaldehyde (MPA) formed from the periodate oxidation of guaiacol glyceryl ether (GGE) were used for the GC determination of GGE in aqueous solutions [28]. Samples were analyzed by GC–FID using packed glass columns (3% XE-60). Experiments on the effect of pH on oxime formation showed that formaldehyde and MPA oximes formed over the pH range 3–6. However, lower yields were achieved as the pH approached 6. MS of the MPA oxime showed a base peak at $m/z$ 123. *E*/*Z* isomers of the MPA oxime were observed.

A methanol–catalase system was described by Kobayashi and Kawai [29] for the GC determination of hydrogen peroxide. This system is based on the reaction of hydrogen peroxide and methanol to produce formaldehyde in the presence of catalase and the subsequent formation of the PFBHA–formaldehyde oxime.

Experiments to optimize pH, reaction period, catalase concentration, methanol concentration and the effect of reducing agents were performed using 30 μg of hydrogen peroxide. These experiments showed that pH between 3 and 8, catalase concentrations above 100 U, methanol concentrations between 8.0 and 30% (for 30 μg of hydrogen peroxide) and reaction times between 40 and 120 min could be used. L-Ascorbic acid was used to test the effect of the reducing compound on the system. It was found that there was no significant effect with 5–45 μg per 3 ml of sample solution containing 30 μg of hydrogen peroxide.

Calibration graphs were found to be linear in the range 1.5–60 μg of hydrogen peroxide in 2.0 ml of aqueous solution with FID and 0.006–1.2 μg for ECD. Correlation coefficients for the calibration graphs were 0.9996 for FID and 0.9995 for ECD. Relative standard deviations were 1.39% with FID and 1.28% with ECD. The detection limit for hydrogen peroxide was reported to be 0.001 μg/ml. Blank values were found to be high, corresponding to about 0.05 μg of hydrogen peroxide. Apparently the system was not calibrated using pure analytical standards of the PFB–formaldehyde oxime. Therefore, the reported values for detection limits and calibration represent relative values. Further, no evidence was provided to determine whether formaldehyde had formed from the reaction of hydrogen peroxide and any naturally occurring organics in the aqueous matrix.

In a continuation of this work, Kobayashi and Kawai used the formation of aldehyde products and subsequent PFB oxime formation as a method for the determination of two other enzymatic reactions. In the first system, monoamine oxidase activity was measured [30], while the second was an assay for glucose in serum using measurements of the formaldehyde produced from the glucose oxidase–catalase system [31].

In the glucose oxidase–catalase system [31], a pH between 5 and 6 gave an optimum yield of the PFB oxime. This pH range was important not only for oxime formation but also for glucose oxidase and

catalase activity. The effects of enzyme concentration, methanol concentration, reaction time and reducing compounds were studied; no attempt was made, however, to optimize PFBHA concentrations. Recoveries with glucose-spiked serum were calculated to be 102 ± 2.7%. Although linearity was achieved in the range 20–100 μg, no detection limit was reported for the method. Blank values were reported to be negligible using FID, although the earlier paper indicated high background levels using ECD [24].

The assay of monoamine oxidase [30] took advantage of a similar production of hydrogen peroxide from the initial enzymatic step and the subsequent reaction of hydrogen peroxide with methanol in the presence of catalase to form formaldehyde. The procedure is similar to that used in the other systems, with PFBHA being added to the solution to react with formaldehyde. Although six different amines were used as substrates in the reaction, no indication was given as to possible aldehyde formation and subsequent interference from these products. The precision of the method using these substrates varied between 5.9 and 2.9%. Blank values were again found to be high using ECD.

Because PFBHA is soluble in aqueous solution, it has been found to be a useful reagent for the analysis of enzymatic byproducts. PFBHA was added to solutions containing enzymes and substrates and could effectively scavenge the resulting aldehyde byproducts, with the PFBHA oximes being readily extracted into hexane. The high background levels of formaldehyde in aqueous solutions appears to be a problem common throughout the literature for the analysis of formaldehyde using PFBHA. Work to ruggedize these methods, including an analysis of the effects of many reagents and the use of pure standards to determine absolute recoveries and instrumental response, needs to be undertaken.

## 5. ENVIRONMENTAL APPLICATIONS OF PFBHA

PFBHA has been used in a wide variety of environmental applications, many of which involve the analysis of contaminants found in aqueous systems (Table 4). This is due, in part, to the ease with which PFBHA reacts with carbonyl compounds and the increased sensitivity to ECD that these PFB oxime derivatives display. In developing methods for the determination of carbonyl compounds as indicators of enzymatic reactions, Kobayashi and co-workers explored oxime formation of lower molecular mass carbonyls in aqueous systems (see section 4.3). This work laid the foundation for environmental appli-

TABLE 4

SUMMARY OF PFBHA METHODS FOR ENVIRONMENTAL APPLICATIONS

| Compound type | Matrix type | Detection method | Chromatographic method | Ref. |
|---|---|---|---|---|
| Aldehydes, ketones | Aqueous solution | FID | Packed column GC | 33 |
| α-Keto acids | Aqueous solution | FID, ECD | Packed column GC | 34 |
| Formaldehyde | Clothes | ECD | Capillary GC | 35 |
| Benzophenone | River water, sea water, sediment | ECD | Capillary GC | 36 |
| Formaldehyde | Air | ECD | Capillary GC | 37 |
| Formaldehyde | Air | ECD | Capillary GC | 38 |
| Aldehydes | Exhaust gas | FTD | Capillary GC | 39 |
| Aliphatic aldehydes | River water, sea water | FTD | Capillary GC | 40 |
| Aldehydes, ketones | Ozonated water | ECD, MS | Packed column GC | 41 |
| Aldehydes, ketones | Ozonated water | ECD, MS | Capillary GC | 42 |
| Aldehydes | Ozonated water | MS | Capillary GC | 43, 44 |

cations. Some of this work was described by Kobayashi and Kawai [32] in a review describing fluorine-containing reagents for the GC determination of carbonyl compounds.

In the initial work, Kobayashi *et al.* [33] studied the reaction of PFBHA with a series of thirteen aldehydes and ketones. Samples were analyzed by GC–FID using 2-m packed columns. Four different stationary phases were utilized, each using a different isothermal temperature for elution. Linear calibrations were obtained between 1 and 50 $\mu$g for formaldehyde, acetaldehyde, isobutyraldehyde and diethyl ketone. The reproducibility of the method was found to be 1.48% using isobutyraldehyde. No indication of the reproducibility or the concentrations of other analytes was provided, nor were the effects of the different columns or column temperatures on the method reported.

The reaction was found to be "complete" for aldehydes after 20 min at room temperature, after which the measured values were constant. The effect of reaction temperature was explored using diethyl ketone. Increasing temperature provided only a moderate improvement. It was stated that PFB–isobutyraldehyde oxime was stable when stored at room temperature for a few days.

In a continuation of this work, Kobayashi *et al.* [34] extended the use of PFBHA to the determination of $\alpha$-keto acids in aqueous solutions. Samples of 1.0 ml, containing eight acids, were analyzed using 0.5 ml of PFBHA at 1.0 mg/ml and a reaction time of 30 min. Other conditions were identical with those used above [27], except that samples were also derivatized with diazomethane. *E*/*Z* isomers were detected and the main peak was used for quantification. It was noted that the ratio of the peak area of the second peak to that of the first became smaller with increasing complexity of the R group in the $\alpha$-keto acid, with the ratio being reversed for $\alpha$-ketoisovaleric acid and $\alpha$-keto-$\beta$-methyl-*n*-valeric acid. Nonetheless, it was believed that the ratios of the *E*/*Z* isomers were sufficiently constant for the main peak to be used for analysis.

The reaction was optimum at pH values between 2 and 5 and with a reaction time of 30 min. The extent of extraction of oxime derivatives increased with increasing acidity of the medium. A linear relative response was achieved for the $\alpha$-keto acids studied in the range 0.2–1.0 $\mu$mol in 1.0 ml of aqueous solution, although different slopes were reported, indicating unequal response factors.

In another report on the use of PFBHA for the determination of formaldehyde, Kobayashi *et al.* [35] detected trace amounts (0.80–198.6 ppm) of formaldehyde in clothes. Finely chopped clothes were extracted with water at 40°C for 1 h. After reaction, the mixtures were saturated with sodium chloride, acidified with sulfuric acid and extracted with hexane. GC–ECD was used for separation and detection. It was reported that 5 ppb of formaldehyde in water were detectable.

Benzophenone was determined in water and sediment using PFBHA derivatization and GC–ECD [36]. Benzophenone was extracted from water into *n*-hexane and then purified by silica gel column chromatography and treatment with sulfuric acid. The purified benzophenone was derivatized with PFBHA and determined by GC–ECD. Spiked samples of river water, sea water and sediment were analyzed with recoveries of 70 ± 11, 64 ± 12 and 66 ± 8%, respectively.

Nishikawa *et al.* [37] measured formaldehyde in air by passing 1 l/min of air through distilled water. The free formaldehyde was then reacted with PFBHA. A volume of 0.2 ml of an aqueous solution of PFBHA (0.5 mg/ml) was used for the derivatizations, with a 40-min reaction time at room temperature. The PFBHA–formaldehyde oximes were determined by GC–ECD. The detection limit was 2 ng with a recovery of 94 ± 4%.

Woelfel *et al.* [38] developed a method for the measurement of formaldehyde in workspace air. Using active or passive sampling in aqueous solutions, PFBHA was used to derivatize free formaldehyde. The PFBHA–formaldehyde oxime was then extracted into hexane and determined by GC–ECD. A detection limit of 1 ng/ml was achieved.

In a method developed by Nishikawa *et al.* [39], formaldehyde, acetaldehyde, propionaldehyde and butyraldehyde were detected in exhaust gas and thermal degradation emissions at 14, 10, 67 and 38 ppb, respectively. Gas samples of 2–30 l were collected using impingers connected in series. The impingers contained 10 ml of an ethanol solution containing PFBHA at 0.3 mg/ml. Capillary GC with flame thermionic detection (FTD) was used for analysis.

Baba *et al.* [40] analyzed river and sea waters for

saturated and α-unsaturated aliphatic aldehydes using PFBHA derivatization. Water samples of 500 ml were analyzed by GC with a 5% phenyl–methylsilicone fused-silica capillary column equipped with a flame thermionic detector.

Butyraldehyde, isobutyraldehyde, crotonaldehyde and methacrolein were determined by this method. Recoveries ranged between 77 and 110% with a relative standard deviation of 2.6 and 9.7% for pure water samples spiked at levels between 1 and 4 μg/l. The detection limits were between 0.6 and 0.9 μg/l. Recoveries from river water varied between 90 and 96% and from sea water between 86 and 94%. Full-scan mass spectra of the derivatives were provided.

Yamada and Somiya [41] demonstrated the formation of a series of aldehydes and ketones from ozonized water using PFBHA derivatization and GC–ECD or GC–MS. GC–ECD and GC–MS were performed using 3 m × 3 mm I.D. packed glass columns of OV-17 on Chromosorb W AW DMCS (80–100 mesh). It was shown that formaldehyde, glyoxal and methylglyoxal were major carbonyl byproducts in ozonized water. No information was provided on the detection limits of this method, however, although calibration graphs were generated. Dissolved ozone interfered with the PFBHA reaction. Mass spectra for the PFBHA oximes of acetaldehyde and methylglyoxal were provided, both showing a base peak at $m/z$ 181.

Glaze *et al.* [42] modified Yamada and Somiya's method [41] through the use of capillary column chromatography, the addition of sodium thiosulfate prior to derivatization with PFBHA, the use of a 2-h reaction time and GC–SIM-MS. Detection limits for seventeen aldehydes were determined, ranging between 1.7 μg/l for formaldehyde to 5.1 μg/l for glyoxal. Two of the seventeen aldehydes detected were methylglyoxal and acrolein. High blank levels of formaldehyde were reported, indicating a potential problem with its determination. Full-scan mass spectra of the seventeen aldehydes and acetone were provided.

In a continuation of this work, Cancilla and coworkers [43, 44] addressed the problem of quantification through the synthesis and spectroscopic characterization of a series of straight-chain aldehydes as their PFBHA oximes. Purified oximes were used as analytical standards to determine the absolute instrumental response, recoveries and reaction efficiencies for the use of PFBHA derivatization for the determination of aldehydes in water. MS, NMR, IR and UV data were reported for the oximes of formaldehyde, acetaldehyde, *n*-heptanal, *n*-decanal and the dialdehyde glyoxal.

Conditions of pH, temperature, extraction number and acid type were optimized. It was reported that matrix effects should be considered when determining aldehydes by PFBHA derivatization, that excess of PFBHA must be present and that pure analytical standards should be utilized in quantification studies. Formation of the monoglyoxal derivative was demonstrated using reaction conditions commonly in use for aldehyde detection in ozonized water. This derivative could be mistaken for the PFBHA–acetone or PFBHA–propanal derivatives by MS.

## 6. INSECTICIDAL PFBHA OXIMES

Although this review concerns the use of PFBHA as a derivatizing agent for the trace determination of carbonyl compounds, it should be noted that other applications for PFBHA have been developed. One such application is the formation of insecticidal PFB oximes [45–49]. The cited papers describe the physiological effects of some of these compounds and provide spectral data, such as NMR, which would be useful to investigators interested in pursuing various aspects of PFBHA oxime formation for analytical applications. Johnson *et al.* [46] described the separation of geometric isomers by HPLC. Holan *et al.* [47] reported proton NMR studies of the *E*/*Z* isomers of various oxime O-ether insecticides and the effect of polar solvents on *E*/*Z* ratios.

## 7. CONCLUSIONS

PFBHA has proved to be a valuable derivatizing agent for the analysis of carbonyl-containing compounds, particularly aldehydes. The ease of oxime formation over a wide pH range and the solubility of PFBHA in aqueous solutions have allowed PFBHA to be used for the determination of aldehydes in a variety of biological and environmental matrices. Much work remains to be done to explore other matrices and to optimize the conditions under

which PFBHA can used as an analytical agent.

The effects of chromatographic and analytical conditions on *E*/*Z* ratios of the PFB oximes have not been fully explored. The possibility of changing *E*/*Z* ratios under different analytical conditions brings into question the accurate quantification of the PFB oximes. This is particularly true if only one isomer peak is used for quantification, as different analytical conditions may cause different isomer ratios to be favored. The use of high resolution capillary columns has generally made the separation of *E*/*Z* isomers easier to achieve than the use of packed columns, allowing for changing isomer ratios to be more easily observed. With dicarbonyl species such as glyoxal or succinylacetone, *E*/*Z* isomerism occurs from oxime formation with both carbonyl groups, increasing the number of possible isomers. The existence of multiple isomers makes it essential to understand better the thermodynamic and kinetic behavior of the various isomers under a variety of analytical conditions. Analytical conditions include the effect of pH, solvent type, reaction time and temperature. Instrumental conditions include injector temperatures, column type and temperature and detector response. The lack of commercially available PFB oxime standards has slowed progress of PFB oxime studies in these areas. However, PFB oximes are easily prepared in the laboratory in milligram or greater amounts. Therefore, research into the use of PFB oximes should employ analytical standards.

The continued reaction of PFBHA with aldehydes in organic solvents could pose significant problems to researchers involved in the detection of aldehydes in the environment. This is particularly true for the more volatile aldehydes such as formaldehyde and acetaldehyde, two aldehydes commonly found in air. Another important consideration is the proper storage of PFBHA and its working solutions. Because of the ease with which PFBHA reacts with carbonyl compounds, it can easily become contaminated through the formation of oximes. This, in turn, may lead researchers inadvertently to add already formed oximes to the mixtures under analysis. For this reason, the effect of different storage conditions on pure PFBHA and the working solutions made with PFBHA should be studied. Recrystallization of PFBHA may be required before use to obtain solutions free of oximes.

A number of other fluorinated reagents have been developed for the determination of carbonyl containing compounds. Pentafluorophenylhydrazine (PFPH) is one such reagent found to be useful for a wide variety of applications. Kobayashi and Kawai [32] provided one of the earlier reviews on the use of this reagent and reported that PFB oximes are generally more easily formed than PFPH hydrazones. Further, PFB oximes were found to be much more volatile and amenable to GC separation. Both types of derivatizing agents produced *E*/*Z* isomers, reacted readily at room temperature in aqueous solution and produced derivatives which were easily extracted into organic solvents.

Because PFBHA is such a versatile reagent, there is little doubt that analytical applications using PFBHA will continue to be developed. This is particularly true in areas where there is a need to determine carbonyl containing compounds as derivatives which are stable under a variety of conditions, which form rapidly, are chromatographed easily and are detected at low concentrations.

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