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Review

Solid-phase analytical derivatization: enhancement of sensitivity and selectivity of analysis

J.M. Rosenfeld*

Department of Pathology and Molecular Medicine, Faculty of Health Sciences, McMaster University, 1200 Main St. West, Hamilton, Ontario L8N-3Z5, Canada

Abstract

Analytical derivatizations enhance the sensitivity and selectivity of determinations for organic compounds. Classical techniques are often based on solution chemistry. Most modern sample preparation techniques, however, are based on solid-phase extractions. Solid-phase analytical derivatization bridges this gap and facilitates sample preparation by combining the isolation step with the derivatization. The solid-phase retains both reagents and derivatized analytes and often permits facile separation of excess reagent or selective elution of the desired products. The most recent solid-phase extraction techniques have been used in conjunction with analytical derivatization to automate the analysis. In this review, analytical derivatizations are presented as functional group analysis.

Keywords: Reviews; Derivatization, GC; Solid-phase analytical derivatization; Sensitivity enhancement; Selectivity; Sample handling; Phenols; Cannabinoids; Prostaglandins; Tetrahydrocannabinol

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*Tel.: +1-416-525-9140; fax: +1-416-570-0163.

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1. Introduction

Analytical derivatization converts the analyte into a product with greater stability, superior chromatographic properties or one that can be detected at higher sensitivity [1,2]. It is also a subset of functional group analysis. As such, it confers selectivity on quantitative determination by labeling only those compounds that react with the derivatizing reagent. In some instances, the derivatization is essential to the isolation of the analytes from the matrix.

The chromatographic and detection techniques set constraints on the chemical and physical properties of the derivatives and, therefore, on the reagents used. Gas chromatographic analysis imparts the requirement of thermal stability and volatility. The use of high sensitivity detection methods such as electron-capture detection (ECD) and mass spectrometry (MS) operating in the negative ion chemical ionization (NICI) mode require that the products be electrophores.

Analytical derivatizations are effective but represent at least one additional step in the sample preparation scheme. The reactions can also produce side products that interfere with the analysis. For these reasons, this powerful approach to improving sensitivity and selectivity often is not considered as a primary technique in method development.

One possible resolution to the problems of extra steps and interferences is to carry out analytical derivatizations on solid phases. Derivatization can then occur concurrently with isolation from the matrix or can be carried out after sorption on the solid phase by reaction in-situ. The reagents, derivatives and side products are retained on the solid phase during sorption and derivatization and can, in principle, be selectively eluted after the derivative is formed. Isolation of analytes from an aqueous matrix onto solid phase also eliminates the severe problems of emulsions that can occur with liquid–liquid extraction of organic analytes from an aqueous biological matrix. Sample preparation techniques based on solid-phase analytical derivatization (SPAD) are also amenable to automation. For instance, SPAD can be combined with thermal desorption to fully automate gas chromatographic methods.

The phases used in SPAD are the standard, commercially available materials applied to the isolation of organics by solid-phase extraction (SPE) or high-performance liquid chromatography (HPLC). Accordingly, conditions for the isolation are well defined. Reagents for SPAD are also well known and so the derivatives, side reactions and chromatographic characteristics have already been described. There is sufficient information in the literature to facilitate development of sample preparations based on SPAD. This literature merits consideration when developing an analytical strategy.

This review has several aims. The first is to describe the various applications of SPAD in gas chromatographic methods. The second is to discuss the understanding of reaction mechanisms. Third is to demonstrate where such an understanding can lead to effective exploitation of the technique. In keeping with the concept of SPAD as a subset of functional group analysis, the organization of the review is based on functional groups rather than compound classes or solid phases. This focus is selected because it is the functional group that defines the derivatization chemistry and reagents.

2. Carboxylic acids

Determination of carboxylic acids is a requirement in numerous scientific investigations as well as for many regulatory applications. Biological matrices (plasma, urine, tissue homogenate) contain large numbers of analytes with carboxyl functionalities and this puts a premium on high resolution, making gas chromatography (GC) the method of choice. Studies in physiology, pharmacology and both pharmacological and environmental toxicology require

determination of carboxylic acids from such diverse matrices as natural waters, blood plasma and urine.

Esterification is a common derivatization method for the determination of carboxylic acids [1,3–7,9–13]. This reaction can be affected either by reaction with diazo compounds [3–5] or nucleophilic attack of the carboxylate on an electrophilic center of the derivatizing agent [1,7,8–12,14]. SPAD mediated both types of reactions in the course of sample preparation.

2.1. Diazo reagents in solid-phase microextraction

2.1.1. Esterification with diazomethane

Methylation with diazomethane is perhaps the most facile analytical derivatization. The solution-based reaction conditions are simple and very well defined [3]. The reagent and the solvent, commonly diethyl ether, are both volatile and can be easily removed from the reaction mixture prior to GC. This avoids overloading of the high resolution columns with extraneous material and simplifies concentration of the analytes. Preparation and use of diazomethane carries some risk but reasonably skilled technicians can carry out these procedures safely.

Pan and Pawliszyn [4] and Lee et al. [5] have both studied diazomethane as a reagent for SPAD in the course the extensive work on solid-phase microextraction (SPME). These methods consisted of a three-step process. Carboxylic acids were sorbed onto the silica fiber coated with adsorbent phase. The fiber containing the acids was then exposed to diazomethane for derivatization. Finally, the fiber was inserted into the heated injector port of a gas chromatograph for thermal desorption. The conditions for optimizing the yield of the ester were unchanged from those used to optimize the sorption of organic acids from water. Acidic pH, high salt concentrations and proper selection of the fiber coating all improve yields. The resulting techniques were simple and automated.

2.1.2. Esterification with 1-pyrenyldiazomethane

Formation of pyrenylmethyl esters on SPME fibers is a second derivatization based on the diazo functionality [6]. In this technique, the reagent, 1-pyrenyldiazo-methane (PDAM), was impregnated onto the fiber. Carboxylic acids were isolated from the aqueous phase and derivatized onto the fiber using head space sampling. Finally, the pyrenylmethyl esters were thermally desorbed for gas chromatographic analysis.

Pan et al. [6] developed a model of the mechanism for the reaction between carboxylic acids and PDAM fiber. There was excellent agreement between the predicted reaction rate and that found for sorption/derivatization of the acids in the gaseous phase. The investigators also determined reaction rates for the reaction between PDAM fiber and both propionic and butyric acid aqueous solution. In this latter study, these two carboxylic acids were determined by head space sampling and this presented a more complex problem.

At ambient temperatures, head space sampling for acids from water coupled with derivatization onto PDAM fiber produced low yields of the PDAM esters. This suggested either a slow reaction rate of acids on PDAM fiber or inefficient transfer from the aqueous phase to the fiber. The reaction rate for derivatization with head space sampling, however, increased at elevated temperature. This increase of yield with temperature was greater than that observed when sorption/derivatization of these acids was determined from the gaseous phase alone. The effect was attributed to an enhanced transfer of analyte from the aqueous phase into the head space rather than to an increase in the reaction rate of acids on PDAM fiber. As a result, low pH, ''salting out'' and proper selection of the fiber were again the critical factors in optimizing the yield.

Extraction/derivatization on PDAM fiber is an elegant technique and can be readily automated. There is, however, scope for improvement. Reaction times are lengthy and this reduces throughput. In addition, the injector vials must be heated, requiring more complex instrumentation rather than a simple automated injector. Finally, the PDAM produces a fairly large reagent peak, suggesting the possibility of contamination of the injector port and/or the column.

2.2. Esterification of carboxylic acids by nucleophilic reaction

2.2.1. SPAD on strong anion-exchange resins

Several authors [7–11] described methods for the determination of carboxylic acids in water using a strong anion-exchange phase to sorb carboxylates

directly from aqueous solutions, including complex biological fluids such as urine [10] or fruit juices [11]. Mono- [7–10] and polycarboxylic acids [11] were successfully determined with SPAD on anion-exchange resins. Both pH and ionic strength were determinants of the extraction efficiency. In addition to extracting the carboxylates, strong anion-exchange (SAX) phases immobilized the anion. Such immobilization was particularly useful as drying conditions were required to produce consistently high yields in subsequent derivatizations [8–12].

Both disk [7] and column configurations of the SAX phase were used [8–11]. Disks are often preferred for analysis of the larger volumes [13] that are compatible with environmental applications. In these reports [7–11], however, both configurations could be used to adsorb analytes from volumes of water ranging from 200 ml to 1 l.

The sorbed analytes were derivatized in-situ with methyl iodide or pentafluorobenzyl bromide (PFBBr) using either acetonitrile [7–11] or supercritical CO_2 [8,11] as solvents. Chatfield et al. [8] reported that, in their hands, supercritical CO_2 was a superior solvent for derivatization of these acids. Elevated temperatures (80–120°C) were used to improve the yields, which were in excess of 90% under optimal conditions for methylation of the chlorophenoxy-acetic acids [8,10]. For more volatile and lower-molecular-mass acids (C_2–C_5 acids), yields for pentafluorobenzylation were lower, ranging from 50–66%, with high variability being reported [10].

Although derivatives are well separated from both PFBBr and CH_3I, the presence of these reagents in the final extract can produce a large solvent front in GC–ECD determination. In particular, a relatively large volume (50 μl) of PFBBr was used [10] and this can be detrimental. Dills and Shen [12] discussed interferences arising from analytical derivatization with PFBBr in solution. Their observations regarding reducing the amount of reagent, maintenance of anhydrous conditions, the side reactions of this reagent and optimization methods are pertinent to SPAD as well.

2.2.2. Esterfication with pentafluorobenzyl bromide on organic resins

Reaction with PFBBr has been extensively used for high sensitivity analysis of carboxylic acids [1,12,14]. The highly fluorinated pentafluorobenzyl (PFB) esters have excellent gas chromatographic properties and can be detected at very low concentrations using either ECD or NICI–MS.

Rosenfeld et al. [15] reported SPAD with PFBBr for the preparation of the PFB esters of higher-molecular-mass carboxylic acids ($>C_{10}$) using XAD-2, a styrene–divinylbenzene crosslinked copolymeric macroreticular resin. The high surface area and high adsorptivity allowed sorption of the carboxylate anion by hydrophobic binding rather than by ion-exchange.

Optimization of the conditions focused on reducing the amount of PFBBr without sacrifice of yield or sample preparation times [12] and was dependent upon achieving a homogeneous distribution of the PFBBr on the surface and several methods were studied [15]. The simplest technique for reducing the amount of reagent was counter-intuitive. In this method, 10 μl of PFBBr were diluted in a volume of trichlorethylene (TCE) before adding it to the reaction mixture, which consisted of the aqueous solution of the analytes and the XAD-2, prior to agitation. When the total volume of organic liquid (TCE+PFBBr) was equal to the pore volume of the mass of the XAD-2, the yield was equivalent to that obtained with 50 μl PFBBr impregnated by dropwise addition. This was attributed to a homogeneous dispersion of the reagent, albeit diluted in solvent, throughout the pores and over the surface of the resin.

3. Phenols

3.1. Polar phenols

Acetylation is a typical and frequently used analytical derivatization for the determination of phenols. Acetates of polar phenols can be formed directly in aqueous solution by reaction with acetic anhydride under alkaline conditions and the lipophilic derivatives can then be isolated by standard SPE or SPME techniques [16]. This technique is widely used for the determination of polar phenols in environmental samples. Alternatively, polar phenols can be isolated on SAX resins and then derivatized by SPAD [17]. Comparison of the two techniques

demonstrated that although the reaction in the aqueous phase is slower, yields are more consistently higher and precision is better. The reduced yields on SPAD were particularly marked for polar phenols at lower concentrations and when 1 l of water was analysed. This was attributed to breakthrough rather than to lack of reaction. Analysis of larger volumes of water is important in decreasing detection limits for environmental analysis. Resolution of the breakthrough problem would expand the applications of SAX resins to environmental analyses.

3.2. Lipophilic phenols — the cannabinoids

Higher-molecular-mass phenols are more readily sorbed and do not require derivatization to lipophilic products prior to isolation from an aqueous phase. Often, however, derivatization is required in order to produce thermally stable products with electrophoric properties. Determination of cannabinoids is an example of this type of analytical problem. These terpenoid phenols possess no functionality that permits high sensitivity detection but require derivatization with PFBBr for detection of the ng/ml concentrations commonly found in plasma after ingestion of marijuana.

Smoke from marijuana cigarettes or from hashish pipes contains only one pharmacologically active compound, Δ^{-9} tetrahydrocannabinol (Δ^{-9}-THC) [18]. After ingestion, Δ^{-9}-THC is converted into a very short-lived active metabolite, 11-hydroxy-Δ^{-9}-THC and, finally, to a urinary metabolite, 11-nor–9-carboxy Δ^{-9}-THC (carboxy Δ^{-9}-THC) [19]. The greatest interest is in the determination of Δ^{-9}-THC from plasma and of carboxy-Δ^{-9}-THC from urine. The former is the active compound and detection of the latter in urine is the test for assessing ingestion and/or abuse of marijuana [19].

Sample preparation of plasma for the analysis of Δ^{-9}-THC was more complex than expected. Lipoproteins in this matrix bound the lipophilic Δ^{-9}-THC and hydroxy Δ^{-9}-THC with sufficient avidity so as to substantially reduce the yield and rate of sorption onto XAD-2. The sorption could be substantially increased by the addition of 100 μl of acetonitrile to 1 ml of plasma [20]. The organic modifier displaced the analyte from the lipoprotein and resulted in a recovery of >90% for both Δ^{-9}-THC and 11-hydroxy Δ^{-9}-THC. The rate of sorption, however, remained slow, with 1 h being required for >90% recovery of Δ^{-9}-THC. The rate of sorption from plasma for the more polar 11-hydroxy Δ^{-9}-THC was faster than that of the parent compound and this reflected the lower avidity of protein binding. Subsequent to sorption, the phenolic group was derivatized with PFBBr under alkaline conditions (0.1 *M* NaOH). With the exception of the pH of the aqueous phase, reaction conditions were identical to those for pentafluorobenzylation of carboxylic acids, including the use of TCE as a diluent. The PFB derivatives were detected at low concentrations using either GC–ECD or GC–NICI–MS.

The alkaline conditions resulted in the derivatization of all organic acids normally found in plasma. If, however, oxygenated solvents such as alcohols rather than TCE were used as the diluent, then yields of PFB esters were substantially reduced [21] and the chromatogram was simplified. In the case of alcohols with 1–5 carbon atoms, yields of the PFB esters varied inversely with the molecular mass of the diluent. With pentanol as the diluent, the magnitude of those GC peaks attributable to fatty acids in the plasma was reduced by 80%, with marginal reduction in the peak arising from Δ^{-9}-THC. The reaction then became specific for phenols in the presence of carboxylic acids. This selectivity was further studied in the derivatization of the carboxy-Δ^{-9}-THC (see below).

4. Carbonyls

Determination of carbonyls has impact on almost every aspect of pharmacology, physiology and toxicology. Analytes can be as simple as formaldehyde or as complex as prostaglandin E_2 (PGE_2). Matrices requiring analysis range in complexity from air [22] to water [23] to blood plasma [24] to urine [25]. The analytical problems are also various. Isolation of formaldehyde from any matrix requires that it be trapped as an involatile derivative [26]. In contrast, PGE_2 can be readily isolated from an aqueous matrix but the 9-keto group must be derivatized in order to stabilize the labile 9-keto, 11-hydroxy structure for analysis by GC [14,27].

Two methods for the determination of carbonyls in air are classical applications of SPAD. They are sufficiently well characterized as to be part of regulatory procedures [28,29]. Both HPLC [28] and GC techniques [29] are accepted by the US Environmental Protection Agency (EPA) and by the US National Institute on Occupational Safety and Health (NIOSH). Although these methods were developed quite some time ago, the problems of carbonyl determination are still under active investigation and new SPAD techniques continue to be reported.

4.1. Derivatizations with pentafluorobenzylhydroxylamine

4.1.1. SPME/SPAD

Carbonyl targeting reagents were impregnated onto SPME to serve as a trap for the isolation and derivatization of formaldehyde from water and air [26]. Several different reagents were tested and pentafluorobenzylhydroxylamine hydrochloride (PFBHA) was preferred. The reagent reacted with the volatile analyte to form the stable PFB-oxime, which was retained on the fiber. The derivative was thermally desorbed onto the column for determination by capillary GC.

SPME has been a very innovative development in SPE and it is interesting to see its applications being expanded to SPAD. This technique also exhibits the advantages of automation and solvent-less injection. Further investigations, nevertheless, could still prove useful. The equilibrium and kinetically restricted reactions result in incomplete derivatization and extraction of the analyte. Sensitivities are, therefore, not as high as they might be with higher yields for extraction and/or derivatization.

4.1.2. Derivatizations on cartridges with flow-through techniques

Nawrocki et al. [30] reported SPAD of aldehydes in water with C_{18} cartridges impregnated with PFBHA (PFBHA-C_{18}). The sample consisted of 50 ml of an aqueous solution containing either known concentrations of specified carbonyls or the carbonyl by-products from ozonolysis. Analytes were extracted and derivatized by flowing the sample through the PFBHA-C_{18}. The derivatives were eluted and concentrated prior to GC analysis. Optimization studies on the reaction conditions reduced the amount of PFBHA to 2 mg. The authours also reported a comparison between SPAD with PFBHA/C_{18} cartridges and liquid–liquid extraction (LLE). The former technique provided a higher yield. This work demonstrates some potential advantages of SPAD over standard methods as well as providing an example of optimization.

4.1.3. SPAD on XAD-2 in a batch process

The determination of carbonyls by SPAD from 1 ml of plasma matrix was based on XAD-2 [31]. The reaction was carried out by agitating the acidified reaction mixture for 20 min and eluting the PFB-oximes in the standard manner. Under these conditions, both aldehydes and ketones were isolated as their PFB-oximes in greater than 80% yield from the resin. as the PFB-oxime. The 20 min reaction represented at least a 17-fold decrease in sample preparation time for the isolation and derivatization of ketones preparatory to GC determination [19,21]. High yield at markedly reduced reaction times, however, required 2–4 mg of PFBHA in the reaction mixture, whereas aqueous phase derivatization required only 0.5 mg [22–25] for derivatization from similar volumes.

Breckenridge et al. [31] reported that a two-step reaction sample preparation reduced the amount of PFBHA without affecting sample preparation time. The first step was a 10-min reaction between PFBHA and carbonyls in the aqueous phase. This solution was then brought into contact with and agitated with the XAD-2 for an additional 10 min to complete the reaction. In the two-step process, yields greater than 80% were achieved with only 0.5 mg of PFBHA.

Under the two-step reaction, however, acetone can react with 0.5 mg of PFBHA in the acidified aqueous phase. As noted above, the rate of formation for the PFB-oxime of acetone in the aqueous phase requires 12–24 h [22–25]. In principle, therefore, after 10 min, only a very small portion of the acetone in the aqueous phase would have been converted to the PFB-oxime. In the second step, the 0.5 mg of PFBHA would not have provided an optimal loading of reagent onto the resin to allow reaction with acetone. The reaction rate in the heterogeneous system would also have been insufficient to provide a high yield in the additional 10 min of agitation

required in step 2. Again, substantial amounts of acetone would remain in the aqueous phase. Subsequently, the acetone would have been lost when the resin and aqueous phase were separated. Accordingly, there would have been no opportunity for the acetone to react with the sorbed PFBHA during work-up. Despite these considerations, the two-step reaction using 0.5 mg of PFBHA provided the same yield of the PFB-oxime of acetone did using 2–4 mg of reagent in the one-step reaction.

4.2. Hydrazone formation

Reaction of carbonyls with 2,4-dinitrophenylhydrazine to form the corresponding hydrazones is the classical derivatization for determination of this class of analyte [29,30]. These derivatives are usually prepared for HPLC analysis but can be determined by GC. They are not, however, sufficiently volatile or thermally stable for high sensitivity GC analysis [32]. An analogous reagent, 2,4,6-trichlorophenylhydrazine (TCPH) was reported to have better reactivity on SPAD as well as superior GC properties [33].

The technique described by Lemphul and Birks [33] was an elegant example of the flexibility of SPAD in dealing with complex analytical problems. In their study, the carbonyls had to be extracted and derivatized in the presence of ozone. This highly reactive molecule oxidizes and inactivates TCPH. Accordingly, a mixed bed reactor was used in which the top part of the cartridge was impregnated with thiosulphate to trap ozone. The bottom part of the cartridge contained the reagent, which was protected from degradation and remained fully reactive to derivatize the carbonyls.

Sample preparation consisted of two steps. In the first air, was pulled through the cartridge. In the second step, the cartridge was taken off line, sealed and heated for 6 min at 100°C to drive the reaction to completion. The derivatives were eluted with acetonitrile and an aliquot was injected for GC analysis. Yields in excess of 80% were reported. Due to breakthrough, the lowest yield was for the recovery of acetaldehyde (80%) followed by a yield of 94% for propionaldehyde. All other carbonyls were recovered in greater than 99% yield.

5. Polyfunctional molecules

5.1. Reactions at nitrogen

A series of polyfunctional amines containing acidic hydrogen were sorbed from water by anion-exchange resins and derivatized on the surface with methyl iodide. Chatfield et al. [8] found that, for a group of such compounds, acetonitrile was superior to supercritical CO_2 for SPAD on anion-exchange resins. Use of the latter solvent produced yields that varied from 50–85%, whereas reaction in supercritical CO_2 the yield varied from 15 to 60%. Use of either solvent, however, produced several methyl derivatives resulting from incomplete methylation of all of the reactive sites. The pattern of derivatives was less complex than that observed for phase transfer catalysis with quaternary ammonium salts which were completely non-selective [8]. This suggests that there are advantages to methylation of amines by SPAD on SAX resins and it is possible that further work may resolve the problem of polymethylation.

5.2. Urinary metabolite of Δ^{-9}-THC

Selectivity of SPAD on XAD-2 was further utilized in the determination of the bifunctional carboxy-Δ^{-9}-THC [34]. The selectivity was controlled by the pH of the alkaline phase as well as by the diluent for PFBBr.

When TCE was used as a diluent, the pattern of reaction products was determined by the pH. At pH 7, the carboxyl but not the phenol was ionized. Only the former functional group, therefore, reacted with PFBBr loaded on XAD-2 and the reaction was selective for derivatization of the carboxyl group in the presence of the phenol. At 0.1 *M* NaOH, both carboxylic and phenolic groups were ionized and the mixed PFB ester/PFB ether was recovered in high yield. If, however, pentanol was the diluent, derivatization was predominantly on the phenolic group despite the fact that the carboxylate ion was also available for reaction. The reaction was, therefore, selective for derivatization of the phenolic moiety in the presence of the carboxyl group on the same molecule. In addition, the underivatized carboxylic acid retained the mono-PFB ether on silica gel with

greater avidity than that for neutral or weakly acidic compounds. This permitted incorporation of a more selective chromatographic step into sample preparation and provided a cleaner isolate preparatory to GC–ECD.

5.3. Prostaglandins

With the exception of the F series, all prostaglandins contain carbonyl and carboxylic acid functionalities that must derivatized for determination by GC [14,27]. All prostaglandins, regardless of the series, also possess hydroxyl groups that must be silylated prior to gas chromatographic analysis. The first two derivatizations have been carried out via SPAD.

Prostaglandin E_2 was isolated from plasma and derivatized at pH 7.4 with PFBBr on XAD-2 [35]. Subsequently, the carbonyls in the sorbate were derivatized in-situ on XAD-2 with methylhydroxylamine (MHA) hydrochloride in pyridine. In recognition of the eluting power of pyridine, the volume of the MHA–pyridine solution was made equal to the pore volume of the XAD-2. Eliminating excess volume of this solvent ensured that there was no elution, and no loss, of analyte from the resin during work up. Since the reaction occurred on the solid phase, excess reagent and pyridine were removed simply by washing of the XAD-2 with acidified water.

In the methoximation reaction, the solid phase functioned simply as a support for the reaction and the subsequent clean-up. Reaction conditions were the same as for solution chemistry requiring ~18 h at ambient temperature or 2 h at 60°C. Moreover, oximation in pyridine can be susceptible to moisture and requires the use of this noxious, high boiling solvent. Wubert et al. [14] reported that replacing pyridine with dimethylformamide reduced the reaction time, but this post-extraction procedure still required the evaporation of a high boiling solvent.

A preferable approach was based on derivatization of the carbonyl as the first step. Rosenfeld et al. [36] demonstrated a direct, simultaneous extraction and oximation of PGE_2 from plasma onto XAD-2 that was complete in 20 min. The reagents were either PFBHA or benzylhydroxylamine (BzHA). Subsequent pentafluorobenzylation of the carboxyl group of the prostaglandins could not be carried out on the resin as the oximes were unstable to the esterification conditions on XAD-2. For this reason, the oximes were eluted and the eluate was evaporated to dryness. The carboxyl groups were then esterified in solution with PFBBr using diisopropylethylamine as a base to ionize the carboxylic acid [14,27].

Benzylhydroxylamine, rather than PFBHA, was the preferred reagent. The NICI mass spectrum of TMS–PFB-oxime/PFB ester of PGE_2 had one predominant peak at m/z 181 resulting from the loss of PFB at the PFB-oxime functionality. Since derivatization was with PFB-containing reagents, the fragment at m/z 181 contained little structural information and this reduced the specificity of the method. In contrast, the predominant fragmentation of TMS–benzyloxime/PFB ester of PGE_2 resulted from loss of a PFB radical from the ester group and the resulting carboxylate anion (m/z 486) retained the entire nucleus of the PGE_2. Monitoring of the higher mass representing an information-rich fragment improved the specificity of the determination.

While this was not a total SPAD technique, it demonstrated two properties of this reaction. The first was the ability to rapidly derivatize the 9-keto group under aqueous conditions, at room temperature and simultaneously with the sorption step. The second property shows that caution is required in carrying out SPAD. The surface of XAD-2 is a very reactive environment and can sometimes break down labile molecules such as the prostaglandins.

6. Conclusion

Solid-phase analytical derivatization is a potentially useful technique for determining numerous analytes and from almost all matrices encountered by the analytical organic chemist. Recent work by Pawliszyn and co-workers [4,6,26] and Lee et al. [5] has demonstrated its utility with the most current SPE technologies. Elaboration of the mechanisms will assist in optimizing the entire analytical process and will move the field forward. A particularly useful development would be more rapid and efficient SPAD reagents, perhaps by preparing more reactive leaving groups for the electrophilic reagents, such as those containing the PFB moiety that imparts

such high sensitivity. The effects expected from complex matrices such as blood plasma are not overwhelming and can be overcome by simple procedures without sacrificing the flow-through or batch-processing techniques of SPE. Nor should the investigator be reluctant to attempt SPAD for the determination of complex molecules such as the prostaglandins and carboxy Δ^{-9}-THC. With further investigations and elution of reaction mechanisms, SPAD may become a more attractive technique for the development of high sensitivity determination of many important analytes.

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