*Journal of Pharmaceutical & Biomedical Analysis*
Vol. 4, No. 6, pp. 825–834, 1986
Printed in Great Britain

0731–7085/86 $3.00 + 0.00
Pergamon Journals Ltd.

# Direct derivatization of drugs in untreated biological samples for gas chromatographic analysis*

J. VESSMAN, K. E. KARLSSON and O. GYLLENHAAL

*Department of Analytical Chemistry, AB Hässle, S-431 83 Mölndal, Sweden*

**Abstract**: The possibilities to derivatize an analyte directly in the biological sample are reviewed with examples from our own experiences and from the literature. Techniques, such as extractive acylation, alkylation and benzoylation, are frequently used. Improvement of the extractability of the drug from the matrix is a common feature, especially with hydrophilic compounds, where sometimes cyclizing reactions can be employed. Several analytes are reactive or labile in the sample and can be trapped in derivatization reactions *in situ*. In many cases, two-phase reactions lead to milder derivatization conditions (e.g. dealkylation of tertiary amines), which is favourable from a clean-up point of view.

**Keywords**: *Derivatization; gas chromatography; biological sample; extractive alkylation; dealkylation; cyclization; two-phase reaction.*

## Introduction

There are several reasons why derivatization reactions have to be performed before gas chromatographic analysis of drugs. The most important reason is the need for reduction of the polar properties to avoid adsorption of trace amounts on the column. Furthermore, the introduction of tags suitable for selective and sensitive detection has been extensively used. The derivatization reaction is usually preceded by an extraction procedure to isolate the analyte(s) from the sample matrix.

In recent years many attempts have been made to combine the isolation and derivatization steps into one procedure. From the literature analysts are now familiar with reactions such as extractive alkylation and extractive benzoylation.

Derivatization of the analyte in the biological sample without prior isolation has certain advantages, of which an important one is improved extractability of the derivatized polar compound. Stability problems with some labile analytes can be avoided if the reaction is carried out immediately after the sampling procedure. Adsorption of certain analytes to glassware during isolation is also avoided.

*Presented at the "Second International Symposium on Drug Analysis", May 1986, Brussels, Belgium.

One drawback with the formation of a derivative already in the sample matrix is that selective isolation of the compound is made more difficult. Moreover, the reaction conditions have to be validated so that the analyte will not be formed unexpectedly from other compounds, e.g. hydrolysis of glucuronides and sulphates.

The present paper comprises a discussion of some examples of *direct derivatization* of drugs in untreated biological samples. This term includes not only derivatization in the sample matrix followed by extraction but also two-phase reactions where the derivatization takes place in the organic phase while extraction of the analyte is continuing from the aqueous sample.

## Direct Derivatization of Drugs

*Extractive alkylation*

This technique is applicable for the derivatization of acids in the anion form, such as carboxylates, barbiturates and phenolates [1–3]. The anions are extracted as ion pairs to an organic phase where alkylation takes place (Fig. 1).

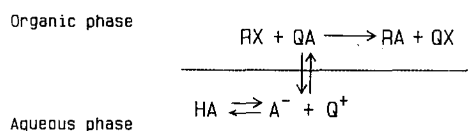


**Figure 1**
Extractive alkylation for the derivatization of acids.

The reaction rate is dependent upon the distribution ratio ($D_{QA}$) given in equation (1). It follows from this equation that the distribution ratio is controlled by the extraction constant ($K_{ex(QA)}$) and the concentration of the counter-ion ($Q^+$). The choice of counter-ion and solvent determines the magnitude of the extraction constant.

$$D_{QA} = \frac{[QA]_{org}}{[A^-]} = K_{ex(QA)_{org}} \cdot [Q^+], \tag{1}$$

where $Q^+$ is the counter-ion, $A^-$ is the acid anion, $K_{ex}$ is the extraction constant and $D_{QA}$ is the distribution ratio of the ion-pair QA.

Even very polar compounds, with a low distribution ratio, can be derivatized as the anion is continuously removed from the organic phase by the alkylation process. The pH in the aqueous phase has to be chosen to give a reasonable fraction of ion-pair in the organic phase, i.e. pH greatly influences the reaction rate at low distribution ratios. The acid in uncharged form does not react at all or at a much lower rate.

The formation of more than one derivative, i.e. di-, tri- and even tetraalkyl- forms, is common among compounds with several labile protons and can complicate the procedure, when the reaction conditions are crucial to control [4].

The use of extractive alkylation directly in the biological sample has been described in several papers. The problems that generally restrict its use are associated with the presence of numerous other anions that compete with the analyte for the counter-ion. The more hydrophobic the counter-ion, the smaller are the anions that will be extracted.

Even inorganic ones like the chloride ion are easily extracted. At high pH, using very hydrophobic counter-ions, such as tetrahexylammonium (THA), the hydroxide ion is also extracted and this can cause hydrolysis of some derivatives as esters. The presence of hydroxide ions in the organic phase also leads to consumption of the alkylating reagent, a decrease of pH in the aqueous phase and a reduction of the available concentration of $Q^+$. The effect will be a lower distribution ratio of the analyte, which might result in a lower reaction rate.

The presence of numerous interfering anions in the biological sample matrix, which also can be alkylated, reduces the possibilities to quantify trace amounts. This can partly be avoided by a selective detection device. In favourable cases with electron capture, or thermionic detection, the use of reagents that do not confer response to the derivative in the detector can result in methods with low limits of determination. This requires that the analyte already has a unique property for the detector: electrophore group for electron capture detection (ECD); or nitrogen for alkali flame ionization detection (AFID). Single-ion monitoring in gas chromatography–mass spectrometry (GC–MS) does not usually have this limitation but the choice of the derivatization reagent can still be important if the fragmentation is not favourable.

Where there are two consecutive derivatization reactions, e.g. for amino-acids, it is very often preferable to use an extractive alkylation procedure as the second one. In that way, the partitioning properties of the intermediate can be utilized for purification and enrichment purposes.

*Examples of extractive alkylation directly in the matrix.* Valproic acid has therapeutic concentrations in plasma in the 50–150 $\mu g\ ml^{-1}$ range, thus making flame-ionization detection possible. Gyllenhaal and Albinsson used extractive methylation successfully with 100 $\mu l$ of serum for the determination of valproic acid [5]. Phenacyl valproate has been shown to have electrophore properties which permitted analysis in 100 $\mu l$ of serum [6]. A crown ether was used as a "catalyst", i.e. assisting in the extraction process. Although a prior protein precipitation was undertaken, the procedure was simple and straightforward.

Ketoprofen, an acid with anti-inflammatory properties, has been quantified after extractive methylation with tetrabutylammonium (TBA) as the counter-ion [7]. A related agent, indomethacin, has also been derivatized directly in the sample with extractive alkylation [8]; in this case, ethyl iodide and THA were used. Both these acids were quantified with ECD using their inherent electrophore properties. Interference from other acids was less pronounced with this approach in which "transparent" reagents were used.

Clioquinol, a chlorophenol derivative, has been determined after extractive methylation in plasma samples (<0.1 ml) [9]. The use of TBA (at pH 11) was shown to be preferable to more lipophilic counter-ions, mainly owing to better stability of the derivative and the reagent.

Diphenylhydantoin (phenytoin) and some of its phenolic metabolites were determined in urine, plasma and various tissue homogenates with an extractive butylation procedure [10]. Using alkaline conditions (~6 M NaOH) and THA the chromatograms, with flame-ionization detection (FID), showed many products in the early part before the diphenylhydantoin derivative. With GC–MS all analytes were clearly distinguishable.

Acetazolamide is a sulphonamide diuretic with three labile protons, of which the amide is a very weak acid. It was quantified in serum (0.1 ml) using an extractive

methylation procedure with tetrapentylammonium (TPeA) as counter-ion [4]. The reaction time was 1 h. A rather high hydroxide concentration (0.5 M NaOH) was necessary to obtain the trimethyl derivative. A lower hydroxide or TPeA concentration gave some di-derivative, indicating that the hydroxide ion concentration in the organic phase is of importance to form the tri-derivative. ECD was used for this electrophore compound. The method could quantify 0.5 $\mu g\ ml^{-1}$, whereas lower levels would require a work-up procedure.

Pentafluorobenzyl bromide is a very useful electrophore reagent and is also very reactive. Numerous papers have been published on its use but in only a few was the reagent used directly on the biological sample. Ritalinic acid, the major amino-acid metabolite of methyl phenidate, has been quantified in urine (0.05–2 $\mu g\ ml^{-1}$) using TBA and pentafluorobenzyl bromide (PFB-Br) [11]. The reaction mixture was purified using various extraction procedures since the derivative is a secondary amine. Yet there were many interfering peaks in the electron-capture gas chromatogram. With TPeA as a counter-ion the chromatograms contained many more interfering peaks.

6-Mercaptopurine was quantified with GC–MS as its bis-PFB-derivative prepared directly from the plasma sample (buffered to pH 10) with TBA as counter-ion [12]. The main advantage was the improvement in extraction selectivity of this very polar compound compared with earlier procedures, using butanol or ethylacetate–isopropanol as solvents. The reaction conditions were optimized with respect to the derivative stability as well as to the selectivity of the assay, i.e. methylation would lead to interference from the *S*-methyl compounds.

Morphine, a phenolic alkaloid, was quantified in a procedure that involved pentafluorobenzylation in ethyl acetate using TBA as counter-ion [13]. Ethyl acetate was chosen as protein precipitation was less pronounced than with the more common halogenated solvents. Quantitation was achieved with GC–MS down to about 5 $ng\ ml^{-1}$.

*Arylation*

Dinitrophenylation of amino-acids has been used extensively although the gas chromatographic properties of the derivatives are less good. A more volatile derivative has been described for the gas chromatographic analysis of tranexamic acid, a synthetic amino-acid [14]; fluoronitrobenzotrifluoride was used in a mixture of the plasma sample and dimethyl sulphoxide buffered to pH 9.4. Arylation proceeded smoothly in this medium, from which the carboxylic acid was extracted and then alkylated in the extractive mode before ECD. The major advantage in this case was an improvement of the extraction characteristics.

*Acylation*

The use of acylation reactions is widely applicable in the analysis of compounds, containing, e.g. amino- or hydroxy-groups as well as combinations of these groups in the same molecule.

*Anhydrides* have been used frequently in the analysis of biologically important compounds such as the arylalkylamines. The procedure of Welsh for the isolation of adrenaline has been modified for analytical purposes at trace level. This reaction in aqueous media with acetic anhydride, in the presence of solid sodium bicarbonate, does not include alcohol groups. In a procedure presented by Martin and Baker the acetamides formed were further derivatized with a perfluorinated anhydride, either for

ECD or GC–MS [15]. A more direct approach was recently presented by Beck and Faull who analyzed metanephrine and normetanephrine as pentafluoropropionyl (PFP) derivatives in cerebrospinal fluid [16]; the acylation reaction took place at 0°C in an ice-bath. The alcohol groups were then derivatized with pentafluoropropionyl anhydride (PFPA) in a subsequent anhydrous reaction before the final analysis with GC–MS in a chemical ionization (CI) mode. It was possible to analyze metanephrine in the nmole $l^{-1}$ range with the use of deuterated internal standards.

A similar approach was used for the isolation of the catecholamine metabolite hydroxymethoxyphenyl ethylene glycol as propionyl derivatives [17]. In the final derivative for GC–MS the propionyl groups were displaced by *tert.*-butyldimethyl (t-BDM) silyl groups.

*Chloroformates* can, in addition to the compounds covered by the acyl anhydrides, be used for the reaction with tertiary amines (see under Dealkylation of Tertiary Amines).

The reaction of catecholamines with chloroformates in buffers of pH 7.4 gives stable carbamates from the amine and carbonates from the phenols [18]. However, the aliphatic hydroxyl group is left unreacted. Trimethylsilylation of the alcohol in the isolated derivative gives a final product with good chromatographic properties but with an unfavourable mass-fragmentation pattern.

De Jong and Cramers [19] performed the initial reaction at pH 7.2, but an improved yield was obtained for secondary amines if, after 5 min, the pH was shifted to 9 and another addition of the reagent was made. Furthermore, they used a t-BDM silyl reagent under such conditions that all carbonate groups were exchanged for t-BDM silyl ones, forming a single and defined derivative. The fragmentation pattern for this *N*-carbamate was favourable allowing determination limits (GC–MS) in the same range as for liquid chromatography (LC) with electrochemical (EC) detection (limit of detection ~50 pg $ml^{-1}$). Compared with LC–EC the latter procedure was admittedly more tedious.

The analysis of amino-acids with work-up procedures using chloroformates has been found to be very useful. Thus 4-aminobutyric acid was determined in cerebrospinal fluid (buffered to pH 8.6) with trichloroethyl chloroformate as reagent for the amino group [20]. The excess of the reagent was removed with dichloromethane, whereupon an extractive methylation of the carboxylic acid followed with TBA as counter-ion. Quantification down to 10 ng $ml^{-1}$ was achieved with capillary chromatography and ECD. The reaction conditions with trichloroethyl chloroformate were evaluated from a kinetic model which took into consideration the consumption of the reagent.

Reactive metabolites of acetaminophen (paracetamol) have been shown to bind covalently to plasma proteins. To verify the structure of the product from the residues of the protein hydrolysates, an efficient isolation procedure was necessary [21]. The suspected *p*-aminophenol cysteine product was treated with ethyl chloroformate to yield an *N,N,O*-trisethoxycarbonyl derivative, which, after acidification and extraction into ethyl acetate, was methylated before direct probe MS [21]. This method was extended by a novel silylation reaction (tBDMS) on the chromatographically (LC) purified ethoxycarbonyl derivative of acetaminophen yielding a cyclic structure amenable for GC–MS [22].

Another very hydrophilic amino-acid is taurine, (2-aminoethanesulphonic acid). The gas chromatographic analysis needs a derivative that protects both functional groups. A recent procedure mixed 0.1 ml urine with internal standard (3-amino-1-propane-sulphonic acid), strong alkali (0.5 M) and water before reacting it with isobutyl

chloroformate for 5 min [23]. The product was isolated as an ion-pair with THA into methylene chloride, whereafter conversion of the sulphonate via thionyl chloride to dibutylsulphonamide took place. There is no need for prior clean-up of the sample.

*Pentafluorobenzoyl chloride* has been used by Delbeke *et al.* [24] for some primary and secondary amines. They named the procedure "extractive benzoylation". Urine or plasma were buffered to pH 9.4 (ammonia). Cyclohexane saturated with triethanolamine was added, followed by the electrophore reagent, pentafluorobenzoyl chloride in cyclohexane. The reaction was completed in less than 5 min. The removal of excess reagent was also achieved in the two-phase procedure. Another application using this approach was described for butanilicain [25]. The excess reagent could not be removed completely to allow ECD here. Instead nitrogen selective detection was used.

*Cyclization*

Phosgene has been used as a cyclizing agent for the amino-alcohol chain of beta-blocking drugs. The results have so far been very promising, with applications for alprenolol and metoprolol as well as for their metabolites [26–28]. The procedure with derivatization of metoprolol in a two-phase system is very simple and straightforward [26]. Nitrogen selective detection was used. The cyclized product, an oxazolidinone, has good chromatographic properties and is much more stable than the trifluoroacetyl (TFA) derivatives usually employed for beta-blockers with ECD.

The presence of additional functional groups in the beta-blockers (or their metabolites) might sometimes restrict the possible use of this reaction. Gyllenhaal studied the influence of the additional groups, alcohol, phenol, carboxyl, etc. [27]. With a phenol group the first product is a chloroformate, which has to be reacted further owing to its reactivity. An alcohol is added and a carbonate is formed. The extractability of amino-alcohols as derivatives is not much improved since the oxazolidinones are rather polar. On the other hand, with metabolites such as "metoprolol acid" (an amino-acid) the situation is improved as the cyclic derivative, an acid, can now be extracted at low pH [28].

The oxazolidinone structure has been shown to be useful on a chiral capillary column in the separation of the enantiomers of beta-blocking drugs [29] and, in combination with methylation of phenols, catecholamines [30]. An application on biological samples with the amino-acid metabolite of metoprolol has been described by Gyllenhaal *et al.* [31].

As shown for the beta-blockers the reaction with phosgene to form an oxazolidinone is very favourable. Another amino-alcohol cyclization has been described for phenylpropanolamine using pentafluorobenzaldehyde [32]. The two-phase reaction took 1 h at room temperature directly in plasma to which the bisulphite adduct of pentafluorobenzaldehyde was added. In the organic phase, hexane and pentafluorophenyloxazolidine were recovered. Excellent response was obtained by ECD.

Other examples where cyclic compounds have been formed in biological samples include alpha-keto acids which are reacted with various phenylenediamines to form quinoxalinols, which are then easily extracted [33, 34]. After a silylation reaction the derivatives can be separated and quantified with different detection principles.

Biguanides and guanidines have been cyclized in reactions that produce either triazines or pyrimidines, respectively. The cyclic compounds have, with certain fluorinated reagents like monochlorodifluoroacetic anhydride (biguanides) [35] or hexafluoroacetylacetone (HFAA) (debrisoquine) [36], given a very strong electron

capture response and have also shown very useful fragments in negative-ion CI GC–MS [37]. The extractability for these compounds is rather low. Hence the two-phase derivatization of debrisoquine with HFAA was an important improvement [36]. So far, the biguanides that react with anhydrides or acyl chlorides have not been shown to undergo two-phase derivatization reactions, but the similarity with other reactions indicates the possibility.

*Dealkylation of tertiary amines*

Chloroformates have been of special interest in the analysis of tertiary amines. This functional group is usually not easy to derivatize. However, under certain conditions, one alkyl group is displaced as an alkyl halide by an aryl- or alkyloxycarbonyl group to form very stable carbamates [38].

The conditions for the dealkylation reaction (especially that of the intermediate quaternary ammonium ion) were chosen after studies based on a model for the two-phase reaction system (Fig. 2). A change from an alkyl to an aryl chloroformate coupled with the addition of iodide ion (cf. $Cl^-$ in Fig. 2) made it possible not only to work at room temperature instead of at 130°C but also with a reagent concentration 200 times lower. A two-phase system with mild reaction conditions can give clean chromatograms even when performed with biological samples. An example of this is the determination of amitriptyline in plasma where the limit of determination was 6 ng $ml^{-1}$ (ECD) [38].

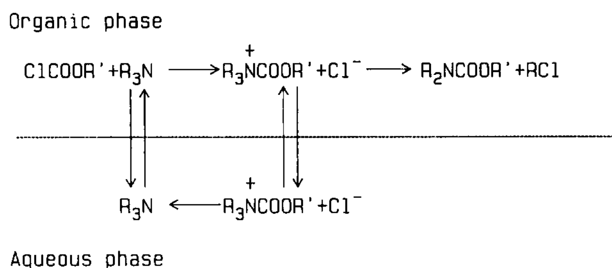


**Figure 2**
Reactions and equilibria involved in the two-phase dealkylation of tertiary amines with chloroformates.

The selectivity in dealkylation reactions has to be carefully evaluated, since secondary amines (as metabolites) often give the same derivative as the corresponding tertiary amine. This problem was solved for amitriptyline by first reacting the secondary amine, nortriptyline, with an alkyl chloroformate followed by a second reaction of the tertiary amine with 2,4-dichlorophenyl chloroformate after the addition of iodide [38]. Excess reagent was removed by treatment with methanolic alkali. With this approach both compounds could be determined from the same chromatogram.

With some tertiary amines it is more advantageous to monitor the alkyl halide instead of the carbamate. Where iodide ion is added to the sample an alkyl iodide is formed, which can be detected easily with ECD. This is an advantage for selectivity as the secondary amine forms another carbamate. This approach was exemplified with *N,N*-diethylnaphthylmethylamine [39].

*Unstable compounds*

Owing to labile structural elements in many analytes, decomposition in the biological sample is not uncommon. Such behaviour will, of course, give varying and erroneous results. Some compounds, e.g. catecholamines, have been shown to be stable in the presence of the biological tissues but, when isolated, the catechol structure is oxidized [40]. The reactions using anhydrides, discussed under acylation, might therefore be suitable to be applied to catecholamines. Derivatization *in situ* has been described for several reactive analytes.

The isolation of the adduct between acetaminophen and cysteine described earlier used chloroformate as protective reagent [21]. This, in addition, rendered the compound less susceptible to autoxidation.

Phosgene can be formed metabolically from chloroform. This very reactive metabolite has been trapped with cysteine in incubation mixtures containing microsomal proteins [41]. The product in this case was 2-oxothiazolidine-4-carboxylic acid.

Captopril is an antihypertensive agent with a sulphhydryl group. This renders the drug sensitive to oxidative degradation. Therefore protection of the mercapto group in a reaction with *N*-ethylmaleimide directly after sampling has been employed frequently [42]. The remaining carboxyl group had to be esterified after extraction of the acid from the treated sample. The sample work-up and isolation procedures were still somewhat tedious. Detection by mass-fragmentography and flame photometry has been used [42, 43]. Recently it was shown that the formed *N*-ethyl succinimide derivative had electrophore properties [44].

Phanquone, an antiamoebic drug with a phenanthroline dione structure, is both unstable and difficult to isolate from the biological matrix. Degen *et al.* utilized an aqueous reaction with methoxyamine that gave a stable and extractable derivative, which in addition had electrophore properties [45]; the detection limit was 15 ng $ml^{-1}$.

Many beta-blockers are metabolically *N*-dealkylated to the corresponding primary amine which in its turn is de-aminated to either a diol or a hydroxyacid. It has been proposed that the latter reaction products are formed via an aldehyde intermediate; this was shown to be extremely labile. Attempts to isolate the aldehyde from the incubates resulted in complete decomposition. However, if methoxyamine was added directly to the incubate it was possible to trap the aldehyde as an *O*-methyloxime. This has been verified for propranolol and oxprenolol [46, 47].

Hydralazine is an antihypertensive agent which is unstable and not easily extractable from biological material with organic solvents. Two methods have been proposed where stable derivatives are formed directly in the biological sample. The first method used a reaction with nitrous acid at acidic pH to form a tetrazolophthalazine, which has good chromatographic properties and electron capture response [48]. Various modifications of this method apparently do not distinguish between "free" and "bound" hydralazine. This led to the development of a second method, which utilized a reaction (<60 min) in plasma with 2,4-pentanedione to form a pyrazolyl phthalazine derivative in about 40% yield [49]. As the reaction was performed at pH 6.4, the risk of reacting acid labile conjugates was considerably reduced. With this method using nitrogen selective detection it was shown that, in fact, only about 10% of total apparent hydralazine was present in the "free" form. It is worthwhile to note that the two methods did give the same results for spiked samples. This observation is important to remember when developing new bioanalytical methods. The equilibria in a real biological sample can never be fully reflected in a spiked sample. Even if LC–EC methods have been proposed

for hydralazine with no sample pretreatment, the problem of instability of the analyte has not been solved in that way [50].

*Displacement of analyte from matrix components*

Primary amino-groups interact with aldehydes to form Schiff bases. Adducts are probably also present in the biological matrix. Such an indication was seen in the analysis of tocainide, a drug with a primary amino group [51]. When hydroxylamine was added to the plasma sample 30 min prior to the extraction, the extraction yield was improved as well as the precision of the assay. Tocainide bound to carbonyl groups is displaced by the high concentration of hydroxylamine, leaving more of the drug available for extraction. This effect was more pronounced in real samples than in spiked ones.

*Limitations of direct derivatization for gas chromatography*

The studies with hydralazine point to one weak point in the use of direct derivatization reactions, i.e. the possibility that conjugates or other transformation products will react or behave like the analyte. The case of secondary and tertiary amines in reactions with chloroformates has been mentioned previously. Another recent example is from the reaction of phosgene with alprenolol and its glucuronides in urine. Gyllenhaal showed that with the conditions chosen [27], the *O*-glucuronide vicinal to the amino-group was displaced by the *N*-chlorocarbonyl intermediate, forming the same oxazolidineone as from free alprenolol.*

## Conclusions

The use of direct derivatization procedures is advantageous as isolation and derivatization are combined in one step. Improved stability of the analyte can also be gained with derivative formation, if necessary already at the time of taking the biological sample. More favourable derivatization conditions can often be used in two-phase reactions. Hydrophilic analytes with several functional groups, i.e. with amino-acid character, can many times be cyclized with, e.g. phosgene, leading to better extractability of the compound. In most cases, the use of direct derivatization procedures requires selective detection principles such as the nitrogen selective detector, electron capture detector, or single ion monitoring, as otherwise too much of the interfering components will be obtained.

## References

[1] A. Brändström and U. Junggren, *Tetrahedron Lett.* **6**, 473 (1972).
[2] H. Ehrsson, *Acta Pharm. Suec.* **8**, 113–118 (1971).
[3] See review by J. D. Nicholson, *Analyst* **103**, 12–14 (1978).
[4] C. Fagerlund, P. Hartvig and B. Lindström, *J. Chromatogr.* **168**, 107–116 (1979).
[5] O. Gyllenhaal and A. Albinsson, *J. Chromatogr.* **161**, 343–346 (1978).
[6] S. C. Chan, *Clin. Chem.* **26**, 1528–1530 (1980).
[7] P. Stenberg, T.-E. Jönsson, B. Nilsson and F. Wollheim, *J. Chromatogr.* **177**, 145–148 (1979).
[8] K. Möller Jensen, *J. Chromatogr.* **53**, 195–202 (1978).
[9] P. Hartvig and C. Fagerlund, *J. Chromatogr.* **140**, 170–173 (1977).
[10] M. Arboix and C. Pantarotto, *Chromatographia* **15**, 509–513 (1982).
[11] M. van Boven and P. Daenens, *J. Forensic Sci.* **24**, 55–60 (1979).
[12] S. Floberg, P. Hartvig, B. Lindström and G. Lönnerholm, *J. Chromatogr.* **225**, 73–81 (1981).

*O. Gyllenhaal, in preparation.

[13] W. J. Cole, J. Parkhouse and Y. Y. Yousef, *J. Chromatogr.* **136**, 409–416 (1977).
[14] J. Vessman and S. Strömberg, *Anal. Chem.* **49**, 369–373 (1977).
[15] R. T. Coutts, G. B. Baker, F. M. Pasutto, S.-F. Liu, D. F. LeGatt and D. B. Prelusky, *Biomed. Mass Spectrom.* **11**, 441–449 (1984).
[16] O. Beck and K. F. Faull, *Anal. Biochem.* **149**, 492–500 (1985).
[17] D. C. Jimerson, S. P. Markey, J. A. Oliver and I. J. Kopin, *Biomed. Mass Spectrom.* **8**, 256–259 (1981).
[18] O. Gyllenhaal, L. Johansson and J. Vessman, *J. Chromatogr.* **190**, 347–357 (1980).
[19] A. P. J. M. de Jong and C. A. Cramers, *J. Chromatogr.* **276**, 267–278 (1983).
[20] N.-O. Ahnfelt and P. Hartvig, *Acta Pharm. Suec.* **19**, 367–380 (1982).
[21] K.-J. Hoffmann, A. J. Streeter, D. B. Axworthy and T. A. Baillie, *Chem.–Biol. Interactions* **53**, 155–172 (1985).
[22] K. J. Hoffmann, A. J. Streeter, D. B. Axworthy and T. A. Baillie, *Mol. Pharmacol.* **27**, 566–573 (1985).
[23] H. Kataoka, S. Yamamoto and M. Makita, *J. Chromatogr.* **306**, 61–68 (1984).
[24] F. T. Delbeke, M. Debackere, J. A. A. Jonckheere and A. P. de Leenheer, *J. Chromatogr.* **273**, 141–149 (1983).
[25] F. T. Delbeke and M. Debackere, *J. Chromatogr.* **237**, 344–349 (1982).
[26] O. Gyllenhaal and J. Vessman, *J. Chromatogr.* **273**, 129–139 (1983).
[27] O. Gyllenhaal, *J. Chromatogr.* **349**, 447–456 (1985).
[28] O. Gyllenhaal and K.-J. Hoffmann, *J. Chromatogr.* **309**, 317–328 (1984).
[29] W. A. König, K. Ernst and J. Vessman, *J. Chromatogr.* **294**, 423–426 (1984).
[30] W. A. König, O. Gyllenhaal and J. Vessman, *J. Chromatogr.* **356**, 354–8 (1986).
[31] O. Gyllenhaal, W. A. König and J. Vessman, *J. Chromatogr.* **350**, 328–331 (1985).
[32] L. Neelakantan and H. B. Kostenbander, *J. Pharm. Sci.* **65**, 740–742 (1976).
[33] U. Langenbeck, A. Hoinowski, K. Mautel and H.-U. Möhring, *J. Chromatogr.* **143**, 39–50 (1977).
[34] L. I. Woolf, C. Hasinoff and A. Perry, *J. Chromatogr.* **231**, 237–245 (1982).
[35] S. B. Matin, J. H. Karam and P. H. Forsham, *Anal. Chem.* **47**, 545–549 (1975).
[36] S. L. Malcolm and T. R. Marten, *Anal. Chem.* **48**, 807–809 (1976).
[37] S. Murray and K. A. Waddell, *Biomed. Mass Spectrom.* **9**, 466–472 (1982).
[38] K.-E. Karlsson, *J. Chromatogr.* **219**, 373–378 (1981).
[39] K.-E. Karlsson and P. Hartvig, *Acta Pharm. Suec.* **18**, 337–348 (1981).
[40] J. Pettersson, E. Hussi and J. Jänni, *Scand. J. Clin. Lab. Invest.* **40**, 297–303 (1980).
[41] L. R. Pohl, B. Bhooshan, N.F. Whittaker and G. Krishna, *Biochem. Biophys. Res. Commun.* **79**, 684–691 (1977).
[42] P. T. Funke, E. Ivashkiv, M. F. Malley and A. I. Cohen, *Anal. Chem.* **52**, 1086–1089 (1980).
[43] Y. Matsuki, K. Fukuhara, T. Ito, H. Ono, N. Ohara and T. Yui, *J. Chromatogr.* **188**, 177–183 (1980).
[44] M. Jemal and A. I. Cohen, *Anal. Chem.* **57**, 2407–2409 (1985).
[45] P. H. Degen, S. Brechbühler, J. Schäublin and W. Riess, *J. Chromatogr.* **118**, 363–370 (1976).
[46] F. Goldszer, G. L. Tindell, U. K. Walle and T. Walle, *Res. Commun. Chem. Pathol. Pharmacol.* **34**, 193–205 (1981).
[47] C.-H. Chen and W. L. Nelson, *Drug Metab. Disp.* **10**, 277–278 (1982).
[48] D. B. Jack, S. Brechbühler, P. H. Degen, P. Zbinden and W. Riess, *J. Chromatogr.* **115**, 87–92 (1975).
[49] P. H. Degen, *J. Chromatogr.* **176**, 375–380 (1979).
[50] K. Ravichandran and R. P. Baldwin, *J. Chromatogr.* **343**, 99–108 (1985).
[51] L. Johansson and J. Vessman, *J. Chromatogr.* **239**, 323–334 (1982).

[*Received for review 28 May 1986*]