FOOD COMPOSITION AND ADDITIVES

Liquid Chromatographic Determination of Residual Isocyanate Monomers in Plastics Intended for Food Contact Use

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A liquid chromatographic (LC) method was developed for the analysis of 10 isocyanates in polyurethane articles and laminates intended for food use. Residual isocyanates are extracted by dichloromethane with concurrent derivatization by 9-(methylaminomethyl)anthracene. The resultant derivatives are analyzed by reversed-phase LC with fluorescence detection. Separation of the isocyanates was studied and optimized. Quantitation uses 1-naphthyl isocyanate as internal standard and standard addition to the food package. Validation demonstrated the method to have good precision (± 2–5%) and recovery (83–95%) for samples spiked with isocyanates at 0.1 mg/kg. The limit of detection was 0.03 mg/kg. Analysis of 19 commercial polyurethane or laminate food packages demonstrated that the method was not prone to interferences. Residues of diphenylmethane-4,4′-diisocyanate were detected in 5 packages and ranged from 0.14 to 1.08 mg/kg.

Organic isocyanates are chemicals in which the electrophilic character of the –NCO group permits easy reaction with molecules containing a nucleophilic center, e.g., water, amines, and alcohols or diols. Urethanes are formed in the reaction with alcohols and diols. If di- or polyisocyanates take part in the reaction, polyurethanes are formed (*see* Scheme 1).

Polyurethane polymers have important industrial applications, e.g., flexible and rigid polyurethane foam coatings, adhesives, and elastomers. Within the food packaging industry isocyanates are used in polyurethane polymers and adhesives. Adhesives are used in polyester or paperboard laminates [e.g., metallized poly(ethylene terephthalate) film laminated to paper as a microwave-interactive "susceptor" material], multilayer high-barrier plastics laminates (e.g., "shelf stable" products), and "boil-in-the bag" laminates. Polyurethane polymers are used for items such as conveyor belts. During manufacture residual unpolymerized isocyanate monomer can remain in the polymer and may migrate into food that subsequently comes into contact with the polymer. Isocyanates are toxic compounds and their health effects are well-documented (1–3). In this paper, we use the term isocyanates to refer collectively to both isocyanates and diisocyanates.

Within the European Community (EC) isocyanates used in the manufacture of plastics materials and articles intended to come into contact with foods are regulated by EC Directive 90/128/EEC and amendments (4, 5). Residual levels in the finished plastic must not exceed 1.0 mg/kg expressed as –NCO. Twelve isocyanates are currently permitted for use in food-contact materials (Table 1). Only 9 of the 12 isocyanates permitted on the EC positive list are commercially available within the EC. Consequently, methodology was developed for the isocyanates available and also for isopherone diisocyanate, which is not on the positive list but does find use in polyurethanes for nonfood applications. We are not aware of any existing method which analyzes combinations of these residual isocyanates in plastics.

The majority of scientific literature concerning isocyanate analysis covers the determination of isocyanates in air in industrial environments (6–11). These methods rely on impingers, coated with a derivatization reagent, to collect isocyanate residues. Derivatization reagents that were used include *N*-(*p*-nitrobenzyl)-*N*′-propylamine (6), 1-naphthalenemethylamine (7), 1-(2-pyridyl)piperazine (8), 1-(2-methoxyphenyl)piperazine (9), 9-(*N*-methylaminomethyl)anthracene (MAMA) (10), and tryptamine (11). Once collected, the final determinative step may be either colorimetric, chromatographic, or polarographic. The use of impingers makes these methods unsuitable for analysis of isocyanates in plastics materials.

Numerous methods were published that detail isocyanate analysis in polyurethane prepolymers (12–14). These methods rely on various derivatization reagents to improve chromatographic resolution and provide a convenient chromophore. One of the most successful derivatization reagents is MAMA (*see* Scheme 2).

The urea derivatives formed with MAMA are fluorescent and have a strong UV chromophore (molar absorptivity, 4×10^5 M/cm at 254 nm). Rastogi (15) developed methodology for the analysis of residual isocyanate monomers in chemical products containing polyurethane or prepolymer diisocyanate. MAMA was used as the derivatizing reagent and detection lim-

Received April 14, 1994. Accepted by AP July 11, 1994.
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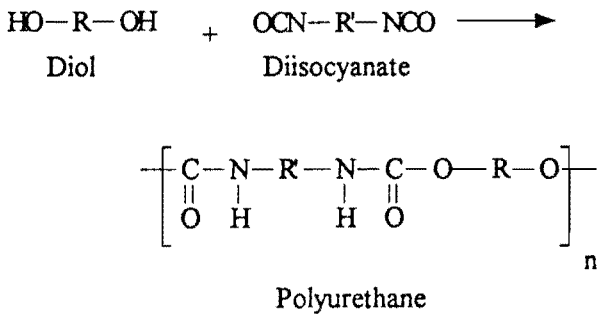


Scheme 1. Reaction of diol with diisocyanate to form a polyurethane of chain length *n*.

its for diphenylmethane diisocyanate (MDI) and toluene diisocyanate (TDI) were 50 and 5 μg/kg, respectively. We report a method applicable for the determination of 10 target isocyanates in plastic materials and laminates by reversed-phase liquid chromatography (LC) following derivatization by MAMA.

Experimental

An instrument or piece of apparatus is mentioned only if it is special or made to particular specifications. Usual laboratory equipment is assumed to be available. All laboratory glassware should be rinsed with dichloromethane (DCM) and baked at 105°C overnight before use. To avoid condensation, after baking vials should be placed in a desiccator to cool until required. The MAMA–isocyanate derivatives are not sensitive to moisture, and so glassware used for operations involving the derivatives need not be so efficiently dried before use.

Apparatus

(a) *Liquid chromatograph.*—Model 305 pump, Model 805 manometric module (Gilson, Anachem, Luton, UK). Model ISS-100 autosampler, Model LS-4 fluorescence detector (Perkin Elmer, Beaconsfield, UK) set at 254 nm (excitation) and 412 nm (emission). Slit widths were set at 10 nm for both excitation and emission.

(b) *LC columns.*—Spherisorb S5ODS2 (25 cm × 4.6 mm, 5 μm), Spherisorb S5ODS1 (25 cm × 4.6 mm, 5 μm), Spherisorb C8 (25 cm × 4.6 mm, 5 μm), Partisil ODSIII (25 cm × 4.6 mm, 5 μm), Zorbax ZODS (25 cm × 4.6 mm, 5 μm), Nucleosil 120 5C18 (25 cm × 4.6 mm, 5 μm), LiChrosorb RP-B (25 cm × 4.6 mm, 5 μm) (Hichrom, Theale, UK). In-line solvent filter, 2 μm (Anachem, Luton, UK). Solvent filter was connected to the analytical column. The analytical column and solvent filter were placed in a column heater (Jones Chromatography, Hengoed, UK) set at 45°C.

(c) *Glass vials (20 mL).*—Chromacol 20-CV (Chromacol Ltd, London, UK). Vials must be rinsed with DCM and baked at 105°C overnight before use.

(d) *Glass amber LC vials (2 mL).*—Chromacol 2-SV(A) (Chromacol Ltd). Vials must be rinsed with DCM and baked at 105°C overnight before use.

(e) *PTFE-faced silicone rubber septa and aluminum crimp caps.*—For 20 mL vials (Chromacol Ltd).

Table 1. Isocyanates on the positive list permitted for use in food contact materials within the European Community

Substance	Abbreviation	Conversion factor[a]
2,6-Toluene diisocyanate	2,6-TDI	0.483
2,4-Toluene diisocyanate	2,4-TDI	0.483
2,4-Toluene diisocyanate dimer	DIMER	0.483
Hexamethylene diisocyanate	HDI	0.500
Cyclohexyl isocyanate	CHI	0.336
Diphenylmethane-4,4′-diisocyanate	4,4′-MDI	0.336
Diphenylmethane-2,4′-diisocyanate	2,4′-MDI	0.336
Diphenylether-4,4′-diisocyanate	DPDI	0.321
1,5-Naphthalene diisocyanate	1,5-NI	0.400
Phenyl isocyanate	PI	0.353
Octadecyl isocyanate	ODI	0.142
3,3′-dimethyl-4,4′-diisocyanato-biphenyl	DIBP	0.318
Isopherone diisocyanate[b]	IPDI	0.378

[a] Factor to convert isocyanates to NCO equivalents (*see* text).
[b] IPDI is not on the positive list but is commonly used for nonfood contact applications.

(f) *PTFE-faced silicone rubber septa and screw caps.*—For LC vials (Chromacol Ltd).

(g) *Crimping device.*—For sealing 20 mL vials (Chromacol Ltd).

(h) *Evaporation unit.*—9 Port Reacti-Vap evaporator with 50 mL/min oxygen free nitrogen flowing through each port, single block Reacti-Therm heating module set at 45°C, and Reacti-Blocks Q-1 and C-1 to hold 20 mL and LC vials (Pierce and Warriner, Chester, UK).

(i) *Glass syringes.*—Type A, graduated (10, 50, 100, and 1000 μL) (SGE, Milton Keynes, UK).

(j) *Laboratory fan-assisted oven.*—Temperature-controlled at 105°C (Gallenkamp, Fisons Scientific Equipment, Loughborough, UK).

(k) *Orbital shaker.*—Model R100 (Luckham, Burgiss Hill, UK).

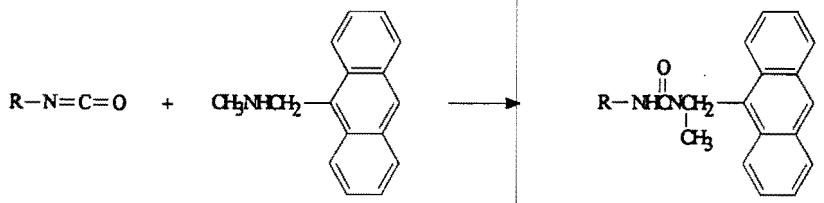
(l) *pH meter and electrode.*—Model PW9418 (Pye Unicam, Cambridge, UK).

Reagents

Caution: Isocyanates are toxic substances. Handling and preparation of standard solutions should be undertaken in a fume hood. Skin and eye contact with isocyanates and inhalation of vapor, should be strictly avoided. Isocyanates react rapidly with moisture. Isocyanate standards should be protected from moisture and stored at 20°C. Precautions should be taken to ensure all glassware is dry.

(a) *Solvents.*—*N,N*-Dimethylformamide (DMF), acetonitrile, and acetone (all LC grade) (Rathburn, Walkerburn, UK). DCM (<30 ppm H_2O) (Rathburn), dried over a bed of 4 Å molecular sieve for 24 h before use; and triethylamine (99%) (Aldrich, Gillingham, UK).

(b) *Standards.*—1-Naphthyl isocyanate (1-NI) (98%), diphenylmethane-4,4′-diisocyanate (98%), hexamethylene di-



Scheme 2. Isocyanate derivatization by MAMA.

isocyanate (98%), cyclohexyl isocyanate (98%), phenyl isocyanate (98%), and isopherone diisocyanate (95%) (Aldrich); 2,6-toluene diisocyanate (99%), 2,4-toluene diisocyanate (99%), and 2,4-toluene diisocyanate dimer (98%) (Bayer, Leverkusen, Germany); diphenylmethane-2,4′-diisocyanate (99%) (Kodak, U.S.); 1,5-naphthalene diisocyanate (98%) (Pfaltz and Bauer, Germany). For other abbreviations, *see* Table 1.

(c) *Molecular sieve.*—4 Å, 1/16 in., 8–12 mesh (Aldrich).

(d) *Orthophosphoric acid.*—1.75 specific gravity (BDH, Poole, UK).

(e) *9-(Methylaminomethyl)anthracene.*—99% (Aldrich).

(f) *LC mobile phase A.*—Acetonitrile–3% triethylamine in water (7 + 3, v/v), adjusted to pH 3.0 with orthophosphoric acid.

(g) *LC mobile phase B.*—Acetonitrile–3% triethylamine in water (3 + 1, v/v), adjusted to pH 3.0 with orthophosphoric acid.

(h) *LC mobile phase C.*—Acetonitrile–3% triethylamine in water (4 + 1, v/v), adjusted to pH 3.0 with orthophosphoric acid.

(i) *LC mobile phase D.*—Acetonitrile–3% triethylamine in water (85 + 15, v/v), adjusted to pH 3.0 with orthophosphoric acid.

(j) *Precipitate dissolution solvent.*—DMF–LC mobile phase C (1 + 1, v/v).

(k) *Polyurethane or laminate samples.*—Samples were obtained from various European suppliers as representative of polyurethane materials for food packaging.

Method Development

Derivatization of standards and LC peak assignment.—Solutions of 1 μg/mL PI; 2,4-TDI; 2,6-TDI; 2,4′-MDI; 4,4′-MDI; HDI; IPDI; PI; 1,5-NI; CHI; and 1-NI (internal standard) were prepared in DCM as working standards. Working standards were protected from moisture and stored at 20°C when not in use. A derivatization solution of 0.24 mg/mL MAMA in DCM was prepared daily because of the photo-instability of MAMA and stored with the exclusion of light. Into 20 mL vials, 100 μL individual working standard and 100 μL derivatization solution was dispensed and vials were capped immediately. Derivatization was allowed to proceed in the dark at ambient temperature for 60 min. The derivatized solution was evaporated to dryness at 45°C under a stream of nitrogen, 10 mL precipitate dissolution solvent was added, and the vial was recapped and shaken vigorously to redissolve the precipitate. A subsample of this solution (1000 μL) was dispensed into an LC sample vial.

Effect of column choice and mobile phase composition.—Derivatized working solutions of 7 isocyanates (PI; CHI; 2,6-TDI; 2,4-TDI; HDI; 2,4′-MDI; and 4,4′-MDI) were analyzed by LC using a variety of columns and LC mobile phases A–D at a flow rate of 1 mL/min. Capacity factors (k) were calculated for each isocyanate derivative peak to determine which column and mobile phase gave the best peak-to-peak resolution.

Effect of derivatization time.—Aliquots (100 μL) of 7 isocyanate working standards (PI; CHI; 2,6-TDI; 2,4-TDI; HDI; 2,4′-MDI; and 4,4′-MDI) and derivatization solution (100 μL) were dispensed into seven 20 mL vials and capped immediately. Individual vials were set aside for 10, 30, 60, 90, 150, 180, and 240 min in the dark. Solutions were prepared for LC as detailed previously and analyzed using a Spherisorb S5ODS1 column and mobile phase C at 1 mL/min.

Effect of extraction time.—Subsamples (1 g) of packaging film NCO19 were cut into 0.25 cm^2 pieces, weighed to an accuracy of 5 mg, and placed into 20 mL vials. To each vial, 15 mL DCM, 200 μL 1-NI working solution, and 1000 μL derivatizing reagent were added. Vials were capped immediately and shaken in the dark for 1, 3, 6, 14, and 24 h on an orbital shaker. Undissolved pieces of film were removed from the vial, and the contents were evaporated to dryness at 45°C under a stream of nitrogen. The evaporated samples were then prepared for LC analysis as described previously and analyzed using a Spherisorb S5ODS1 column and mobile phase C at 1 mL/min.

All extraction time experiments were undertaken in duplicate, and a graph was plotted of isocyanate derivative peak area versus extraction time.

Optimized Procedure

Identification of isocyanates present in test samples.—A 1 g portion of test sample was accurately weighed to the nearest 5 mg, and then cut into 0.25 cm^2 pieces. Pieces were placed into a 20 mL vial, and 15 mL DCM, 200 μL 1-NI working standard, and 1000 μL derivatizing solution were added. The vial was capped immediately. Vials were shaken gently on an orbital shaker for 12 h in the dark. Undissolved pieces of sample were removed from the vial, and the contents were evaporated to dryness at 45°C under a stream of nitrogen. A 10 mL aliquot of precipitate dissolution solvent was added and mixed thoroughly. Ultrasonication may be used to aid dissolution. The solution was filtered through a 0.45 μm syringe filter (prepurged with 2 mL LC mobile phase C) and transferred to an LC sample vial.

Preparation of reagent blank sample.—15 mL DCM, 200 µL 1-NI working standard, and 1000 µL derivatizing reagent were dispensed into a 20 mL vial. The vial was capped and treated as above, leading to sample in an LC vial.

Preparation of internal standard check sample.—A representative 1 g sample was weighed to an accuracy of 5 mg, cut into small pieces where possible, and placed into a 20 mL vial. A 15 mL aliquot of DCM and 1000 µL derivatizing reagent were added. The vial was capped and treated as for test samples, leading to sample in an LC vial.

Chromatographic determination.—Because of the response characteristics of many fluorescence detectors, obtaining a linear response for all calibration solutions may not be possible. In this case the detector must be optimized by decreasing the injection volume or adjusting the slit widths so that the detector is linear over the desired range.

The LC system was equilibrated using a Spherisorb S5ODS1 column and mobile phase C at 1 mL/min. To establish retention times of analytes and the internal standard derivative, 20 µL of each individual isocyanate derivative was injected.

A 20 µL aliquot of sample extract was injected, and the presence of one or more of the 10 isocyanate derivatives was established from retention times. Any isocyanate identified must be used for the standard addition solution preparation. The signal-to-noise ratio for the internal standard derivative must exceed 3:1 to indicate that the derivatization and analysis was successful.

The reagent blank sample (20 µL) and the internal standard check sample (20 µL) were injected. If peaks coelute with those of the isocyanate derivatives, the area of the derivatives should be adjusted accordingly in the final calculation.

Standard addition.—If the presence of isocyanate was indicated, quantitation was carried out by standard addition. A 0.01 g portion of any isocyanate standard(s) identified by screening was weighed to an accuracy of 0.1 mg into a 100 mL volumetric flask. The flask was rapidly made up to the mark with DCM and shaken thoroughly. Ultrasonication may be used to aid dissolution. Into each of seven 1000 µL volumetric flasks, 0, 5, 10, 50, 100, 250, and 500 µL of individual standard addition stock solutions of the isocyanates identified were accurately dispensed. The flask was made up to the mark with DCM and mixed thoroughly.

One-gram portions of a representative sample of the test material or article were weighed to an accuracy of 5 mg and placed into each of seven 20 mL vials after being cut into 0.25 cm^2 pieces, where possible. To each vial, 15 mL DCM, 200 µL internal standard solution, 1000 µL derivatizing reagent, and 1000 µL of each diluted standard addition solution were added. Vials were capped and treated as for test samples leading to the sample in an LC vial. LC was used to analyze 20 µL injections of each extract, to identify the isocyanate derivatives and internal standard derivative peaks on the basis of their retention times, and to measure the respective peak areas.

Data analysis.—Each sample must be determined at least in duplicate. The test sample solution and the samples fortified with isocyanates were used to construct a calibration graph. The graph was a plot of the isocyanate derivative and 1-naphthyl isocyanate derivative peak-area ratio obtained from the standard addition solutions versus the isocyanate concentration added to the test material (mg/kg). Outliers were identified. The isocyanate concentration of the test sample solution was read from the calibration graph by back-extrapolation to the x-axis, where the magnitude of the intercept was equal to the isocyanate concentration. Alternatively, the isocyanate concentration of the test sample solution could be determined mathematically by least-squares regression.

If the internal standard check sample shows an interference in the internal standard region of the chromatogram that exceeds 10% of the area of the internal standard in the calibration samples, and if the analysis of replicate control samples reveals that this interference varies by more than ± 20% in absolute size, then quantitation by external calibration must be used.

If the reagent blank sample shows a peak eluting at the same retention time as the isocyanate derivative, the peak must be quantitated by standard addition omitting the test sample and subtracted from the test sample value that was determined by standard addition.

The concentrations of individual isocyanates should be converted to NCO equivalents by multiplication with the appropriate factor indicated in Table 1. Add NCO values for each individual isocyanate to give total NCO content. This procedure directly yields the isocyanate concentration in the test sample (mg NCO/kg polymer).

Method validation.—A standard mixture (1 µg/mL) of PI; 2,4-TDI; 2,6-TDI; 2,4′-MDI; 4,4′-MDI; HDI; IPDI; 1,5-NI; DIMER; and CHI was prepared in DCM. Exactly 1 g test material was weighed to an accuracy of 5 mg. Ten 1 g portions were placed into separate 20 mL vials, and pieces were cut into 0.25 cm^2 sections where appropriate. Five vials were spiked with 100 µL test mixture, and the remaining 5 vials were spiked with 2.5 mL test mixture. Isocyanates were analyzed as previously described and percentage recoveries were calculated.

Results and Discussion

Method Development

The 7 isocyanates used during method development were chosen to represent the range of isocyanates on the positive list.

Effect of column choice and mobile phase composition.—Figure 1 (I–VI) shows the elution characteristics of the 7 isocyanate derivatives obtained by using commercial LC packings. All the phases examined resulted in the clean separation of the reagent amine and the isocyanate derivatives. Resolution varied greatly depending on the stationary phase. The most variable isocyanate derivative was that of HDI. Using Spherisorb S5ODS1 and S5ODS2 columns the HDI derivative eluted last, whereas elutions using Zorbax ZODS and Nucleosil 120 5C18 columns showed the HDI derivative to elute third and forth, respectively. Elutions using Partisil ODSIII or Zorbax ZODS showed the 2,6-TDI derivative to elute before the 2,4-TDI derivative, whereas elutions using the remaining 4 columns showed the 2,4-TDI derivative to elute before the 2,6-TDI derivative. Using a Nucleosil 120 5C18 column the HDI deriva-

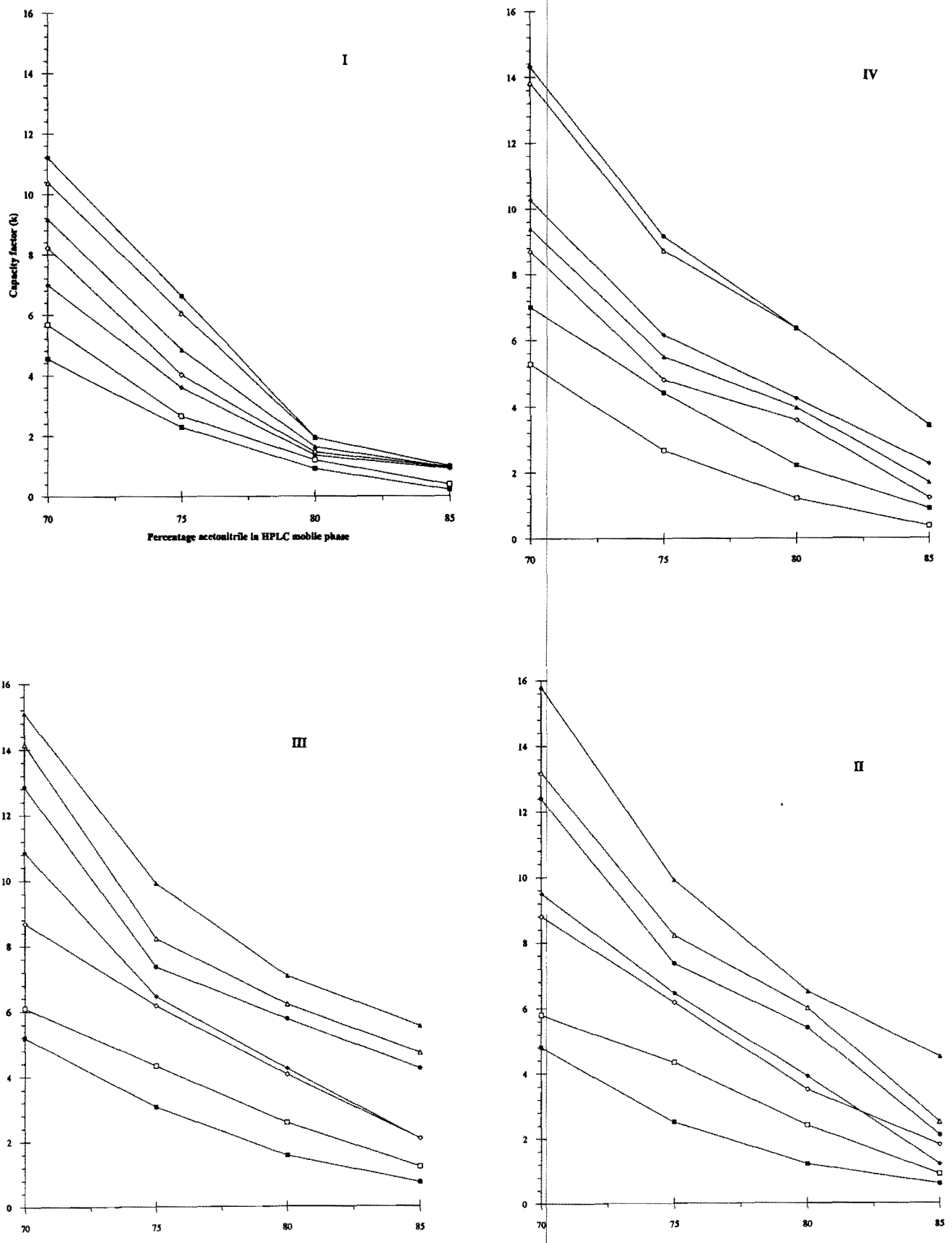


Figure 1. Effect of mobile phase composition on the elution of isocyanate derivatives on various LC stationary phases: I, Spherisorb C8; II, Spherisorb S5ODS1; III, Spherisorb S5ODS2; IV, Nucleosil 120 5C18; V, Partisil ODS3; and VI, Zorbax ZODS.

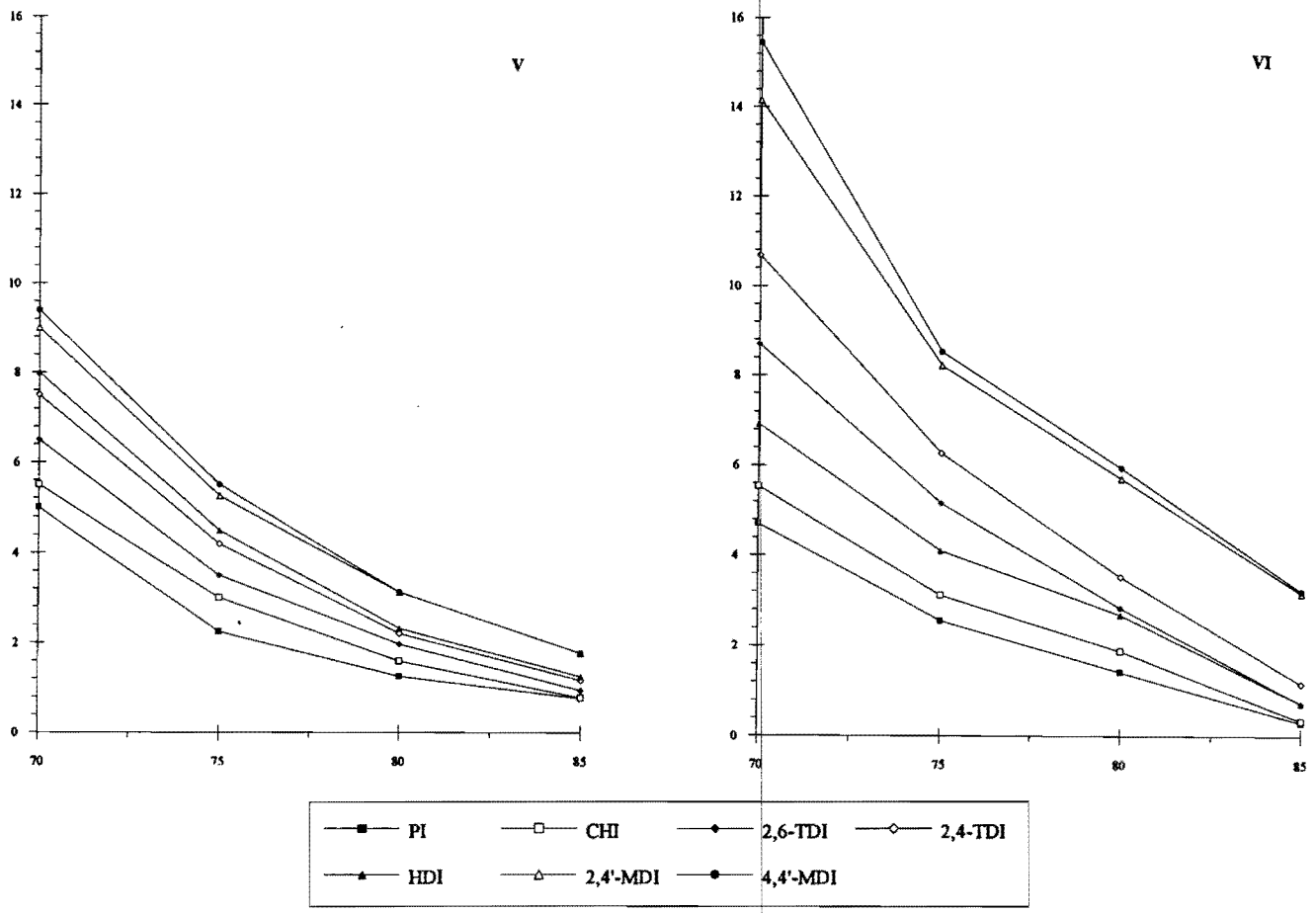


Figure 1. ***(Continued)***

Table 2. Method validation recovery

Sample	PI	CHI	1,5-NI	2,6-TDI	2,4-TDI	2,4'-MDI	4,4'-MDI	IPDI	DIMER	HDI
NCO19, 2.5 mg/kg spike										
Mean, %	89	92	93	87	89	87	91	90	87	88
STD, %	2.3	3.1	2.8	2.9	3.1	1.9	3.4	3.8	2.8	2.1
RSD, %	2.6	3.4	3.0	3.3	3.5	2.2	3.7	4.2	3.2	2.4
NCO19, 0.1 mg/kg spike										
Mean, %	91	93	91	90	87	86	87	88	88	87
STD, %	2.8	2.9	2.4	3.5	4.0	2.4	2.8	3.1	2.8	2.7
RSD, %	3.1	3.1	2.6	3.9	4.6	2.8	3.2	3.5	3.2	3.1
NCO20, 2.5 mg/kg spike										
Mean, %	92	84	86	87	92	91	94	86	88	87
STD, %	2.8	3.4	2.9	3.1	3.5	4.1	2.5	3.6	3.1	2.9
RSD, %	3.0	4.0	3.4	3.6	3.8	4.5	2.7	4.2	3.5	3.3
NCO20, 0.1 mg/kg spike										
Mean, %	92	93	95	83	87	86	84	88	87	89
STD, %	3.5	3.6	2.9	3.8	3.4	3.6	3.1	3.5	3.3	3.5
RSD, %	3.8	3.9	3.1	4.6	3.9	4.2	3.7	4.0	3.8	3.9

Table 3. Polyurethane or laminate samples used for method development and validation[a]

Sample code	Manufacturer	Isocyanate found	Isocyanate, mg/kg	NCO, mg/kg
NCO11	C	4,4′-MDI	0.68	0.23
NCO12	C	4,4′-MDI	0.58	0.19
NCO13	C	4,4′-MDI	1.08	0.36
NCO14	C	4,4′-MDI	0.94	0.32
NCO19	E	4,4′-MDI	0.14	0.05
NCO20[b]	F	IPDI	0.48	0.18

[a] A selection of 20 samples (coded NCO1–NCO20) from 6 manufacturers were analyzed. Fourteen samples contained no detectable isocyanates. Results for the remaining 6 are shown.
[b] NCO20 is not for food use.

tive eluted between the 2 TDI derivatives. It is reasonable to assume that the observed effects reflect differences in the properties of the silica matrix and in the methods of bonding the octadecyl silyl group to the matrix. The type of chlorosilane used, e.g., octadecyltrichlorosilane or an octadecyl silane in which one or 2 of the chlorine atoms have been exchanged for methyl groups, could also influence the properties of the phase, especially the number of remaining silanol groups, which are known to contribute to the chromatographic retention (10).

Standard chromatographic theory stipulates that for optimum resolution, peak symmetry, and analysis time, peak capacity factors (k) should be $1 < k < 10$. Of the stationary phases and mobile phases examined, optimal resolution was obtained with the Spherisorb S5ODS1 column and mobile phase C. Under these conditions, all the derivatives were fully resolved, and capacity factors ranged from 1.8 to 7.6. Full resolution was obtainable with any of the other columns when using mobile phases with lower concentrations of acetonitrile; however, a concurrent increase in capacity factor values and hence lengthy analysis times with isocratic elution resulted.

Effect of derivatization time.—Derivatization time for the 7 isocyanates tested was varied from 10 to 240 min. Derivative peak areas for each isocyanate did not change significantly over the time periods examined. Relative standard deviations (RSDs) over the test periods ranged from 1 to 6%. Higher RSDs were obtained for the PI and CHI derivatives because these derivatives eluted slightly on the tail of the MAMA peak. Because of this tailing, the PI and CHI derivatives were not optimally integrated. Nevertheless, the values were within acceptable analytical limits and indicated good precision. The derivatization is rapid and is complete within 10 min. The isocyanate–MAMA derivatives were stable. Degradation was approximately 1–2% over 3 months when stored in LC mobile phase C, in the absence of light, at 20°C.

Effect of extraction time.—Preliminary analysis of food package NCO19 indicated the presence of 4,4′-MDI residues, so this sample was used in tests to optimize the extraction efficiency. Maximum extraction was obtained after 9–14 h, and between 14–24 h no increase occurred in the amount of 4,4′-MDI extracted. Sample NCO19 was a laminate; hence, the polyurethane–isocyanate, used as an adhesive, was protected by the outer layers of material. An extraction time of 12 h was considered to be sufficient to extract residual isocyanate monomer from laminate materials. For packaging materials in other forms, such as solid polyurethane sheets, we recommend optimization of extraction times as previously mentioned.

Method validation.—Validation data are shown in Table 2. Recoveries from laminate food package NCO19 spiked at 2.5 mg/kg were 87–91%, and RSDs were 2.2–4.4% (n = 3).

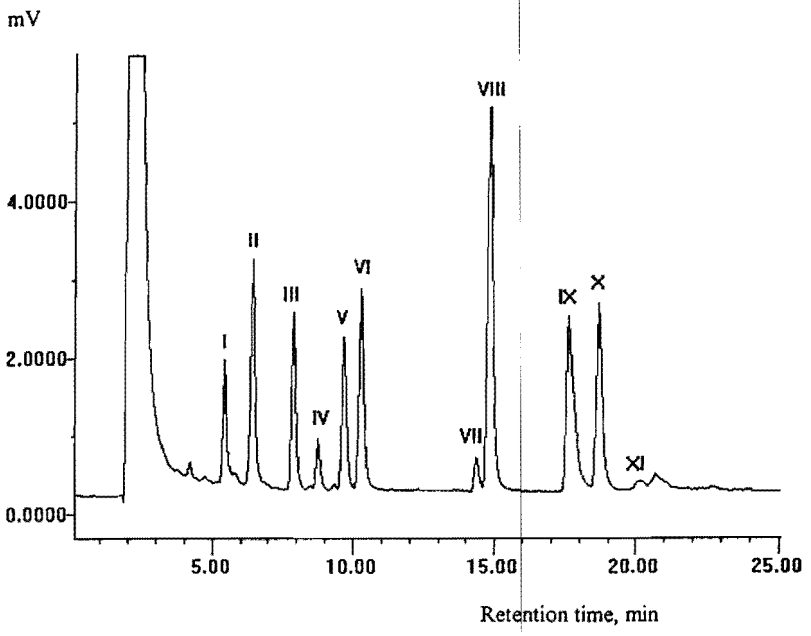


Figure 2. Example liquid trace chromatogram for standard isocyanate derivatives: I, PI; II, CHI; III, 2,4-TDI; IV, 2,6-TDI; V, 1,5-NI; VI, 1-NI (internal standard); VII, 2,4′-MDI; VIII, IPDI; IX, 4,4′-MDI; X, HDI; XI, dimer.

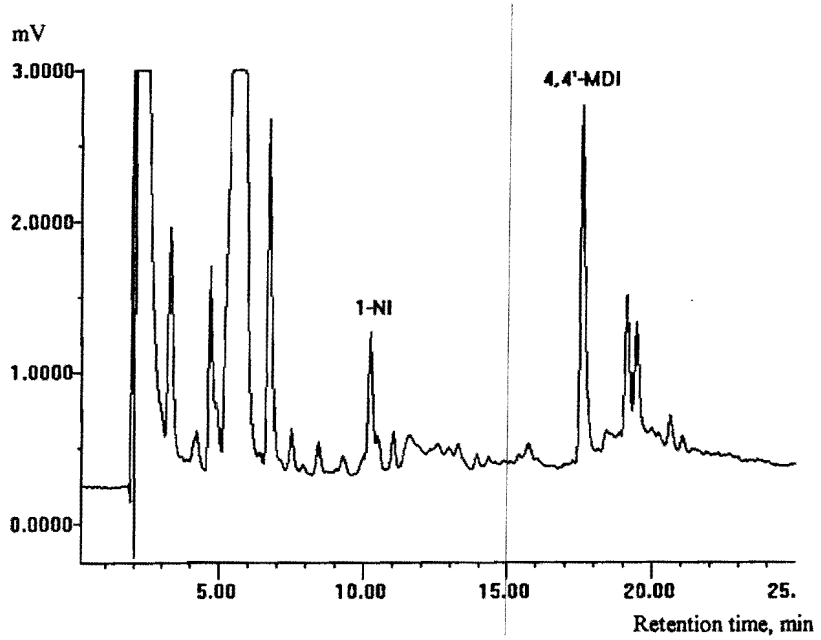


Figure 3. Example of liquid chromatogram for sample NCO13 containing 1.08 mg/kg 4,4'-MDI.

Recoveries from the same sample spiked at 0.1 mg/kg were 86–93%, and RSDs were 2.6–4.6%.

Recoveries from polyurethane sample NCO20 spiked at 2.5 mg/kg were 84–94%, and RSDs were 2.7–4.5%. Recoveries from the same sample spiked at 0.1 mg/kg were 83–95%, and RSDs were 3.1–4.6%. The validation results clearly show that the method will quantitatively determine residual isocyanates effectively extracted from polyurethanes and laminates. The low RSDs indicate good method precision. The mean limit of detection for each of the 10 isocyanate derivatives was 0.03 mg/kg.

Analysis of Commercial Food Packages

The method was applied to 19 commercial food packages (Table 3). The packages were plastic laminates that typically contain a polyurethane adhesive. Five samples contained 4,4'-MDI ranging from 0.05 to 0.36 mg/kg NCO. This range is well below the legislative limit of 1 mg/kg NCO. We also demonstrated that the method could detect isocyanates not on the approved positive list. For this purpose a sample of polyurethane sheet of 2 mm thickness was obtained. This sheeting was not food-contact grade plastic, and IPDI was found at 0.18 mg/kg NCO. IPDI is a common ingredient of polyurethanes outside food contact applications.

Figures 2 and 3 show specimen chromatograms of an isocyanate standard mixture and the extract obtained from food package NCO13, respectively. These results indicate that the method can be applied successfully to commercial food packaging materials without interference.

Conclusions

A method was developed for the analysis of residual isocyanates in food contact materials. Validation demonstrated good method precision. Recoveries from spiked samples ranged from 84 to 95% for the isocyanates analyzed. The isocyanate derivatives formed during analysis exhibited minimal decomposition over a 3-month period. This finding may be useful for enforcement procedures, enabling extract reanalysis by a secondary laboratory or confirmation by reanalysis using different chromatographic conditions. Method development showed that LC column choice is critical if isocyanate derivatives are to be fully resolved from each other in a reasonable time. Spherisorb S5ODS1 columns gave optimum resolution. Derivatization times are not critical. However, we recommend that extraction times of at least 12 h should be used to be certain that all available isocyanate is extracted from the test plastic. Analysis of a small number of polyurethane or laminate materials showed that the method could be applied readily to commercial materials. Residues of 4,4'-MDI were detected in 5 samples and ranged from 0.05 to 0.36 mg/kg NCO.

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FOOD COMPOSITION AND ADDITIVES

Liquid Chromatographic Determination of Vitamin K_1 in Infant Formulas and Milk

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Vitamin K_1 in infant formulas and milk products is determined by reversed-phase liquid chromatography (LC) with UV detection. The sample is hydrolyzed enzymatically, and the vitamin is extracted with hexane. Fractionation by normal-phase semipreparative LC is followed by analytical LC, with quantitation by the internal standard technique. Recovery of the analyte was 97.4 ± 2.8%. Linearity was established between 0.05 and 4.0 μg/mL. The limit of quantitation is 0.5 μg/100 g for milk powder, which allows the method to quantitate endogenous levels of vitamin K_1.

Vitamin K_1 (phylloquinone) is a cofactor in posttranslational modification of calcium-binding proteins involved in antihemorrhagic activity and, more speculatively, in calcium homeostasis (1, 2). Supplementation of infant formulas with vitamin K_1 is intended to protect newborns against hemorrhagic disease. Fortification levels remain controversial, because they significantly exceed typical concentrations of the vitamin in human breast milk.

The physicochemical properties and low levels of vitamin K_1 in biological fluids, combined with the high concentration of lipids in milk, make analysis of this micronutrient in milk challenging. The occurrence of the *cis* isomer in infant formula milks may also be a complication. Earlier data for foods were based on time-consuming biological assays, as well as thin layer and gas–liquid chromatography. These methods have been reviewed comprehensively (3–5) and generally assessed as nonspecific and imprecise.

Liquid chromatography (LC) has superseded other techniques. Several procedures of varying complexity are available for estimation of phylloquinone in milk and supplemented infant formulas (6–15). Initial extraction of vitamin K_1 has been achieved either by exhaustive total lipid partition (7, 10, 11, 13, 14) or after enzymatic hydrolysis of milk triglycerides (6, 8, 9, 12, 15). Further chromatographic purification, including open-column LC or solid-phase extraction (SPE), were required irrespective of the final analytical LC detection technique. LC analysis of infant formulas after enzymatic removal of bulk lipids was successful with UV (8) or fluorescence (9) detection. Further fractionation steps are, however, recommended for analysis of the vitamin in human milk at endogenous levels, prior to analytical LC with more sensitive electrochemical (12) or fluorescence (15) detection.

Highly manipulative extractions and sophisticated detection strategies are successful in small-scale clinical studies, but they are impractical for quality control (QC) of infant formulas. The Association of Official Analytical Chemists has adopted as first action the method of Hwang (10), despite difficulties associated with routine, open-column LC cleanup, absence of internal standard, and formulas containing corn oil (16). For these reasons, variations of the enzymatic procedure have been established (6, 8, 9). Enzymatic hydrolysis may be the easiest procedure, combining minimal sample preparation, instrumental simplicity, and robust UV detection. However, our experience

Received March 29, 1994. Accepted by JL August 3, 1994.