HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF 2,4- AND 2,6-TOLUENEDIAMINE IN AQUEOUS EXTRACTS

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SUMMARY

A method is presented for the determination of 2,4- and 2,6-toluenediamine (TDA) in aqueous extracts of food-contact boil-in-bag and retortable pouches. The extracts are subjected to a methylene chloride extraction clean-up procedure which effects a 50-fold concentration of the amines. A 500-μl portion of the concentrate is analyzed by high-performance liquid chromatography on a reversed-phase, 5-μm, C_8 column. The mobile phase is 10 % acetonitrile in aqueous pH 7.4 phosphate buffer at a flow-rate of 1 ml/min. The lower limit of quantitation is *ca.* 1 ng of TDA on-column or a concentration of *ca.* 40 ng of TDA per liter in the unconcentrated aqueous extracts. Recovery data averaged 89 ± 4 % for standard solutions and spiked extracts over the concentration range 50–2000 ng/l. Results of migration studies are also presented.

INTRODUCTION

Polymeric food-packaging materials contain numerous components that have the potential to migrate out of the polymer matrix and into the food. If any of these components are hazardous to humans, the extent of their migration must be measured and shown to be within acceptable safe limits. Our laboratory wanted to determine whether any 2,4-toluenediamine (TDA) migrates from boilable and retortable plastic food pouches. Toluenediisocyanate (TDI), a component in adhesives used for laminating these products, is readily hydrolyzed to TDA and is the source of its potential migration. Any migration of 2,4-TDA into foods must be closely monitored because animal-feeding studies have indicated that it is carcinogenic[1–3].

Evidence for the migration of aromatic amines at the low parts-per-billion (ppb) (micrograms per liter) level is found in data submitted in manufacturers' petitions for the regulated use of adhesives containing TDI. Earlier studies in our laboratory also suggested such migration. Because the method used to monitor this migration was a non-specific colorimetric technique that responded generally to primary and secondary aromatic amines, we sought a new method which would be both specific and sensitive for quantitating 2,4-TDA at low or sub-ppb concentrations.

Several methods have been reported in the literature that use both gas (GC)

and liquid chromatography (LC) for the analysis of aromatic amines[4–8]. However, none of the reported uses deals with aqueous solutions of TDA at the ppb level, where interactions with "active sites" would be most pronounced. The method reported here employs the technique of ion-suppression high-performance liquid chromatography (HPLC) specifically to minimize these interactions. Consequently, peak tailing is minimized and both sensitivity and resolution are maximized.

We were able to improve sensitivity even more by studying the effect of sample solvent composition on chromatographic peak shape[9]. We achieved minimal peak broadening as the net result while using the exceptionally large injection volume of 500 μl.

The lower limit of reliable quantitation was reduced still further by a methylene chloride extraction procedure which effected a 50-fold concentration and also removed interfering and late-eluting peaks.

EXPERIMENTAL

Equipment

The liquid chromatograph used was a DuPont 850 LC (DuPont, Wilmington, DE, U.S.A.) with fixed-wavelength UV detector, column oven compartment, and a Rheodyne 7125 syringe-loading injector (Rheodyne, Cotati, CA, U.S.A.). LC operating conditions were the following: flow-rate, 1 ml/min; oven temperature, 40°C; UV detector, 254-nm filter, 0.002 a.u.f.s.; injection volume, 500 μl. The UV detector was connected in series to a Spectrum Model 1021A electronic filter/amplifier (Spectrum, Newark, DE, U.S.A.), operated at a frequency cutoff of 0.02 Hz and a gain of either 1X or 2X, and a Hewlett-Packard 7130A strip chart recorder (Hewlett-Packard, Palo Alto, CA, U.S.A.). The reversed-phase column was a 5-μm, Ultrasphere-octyl (Altex, Berkeley, CA, U.S.A.), 250 × 4.6 mm I.D. Other columns were also evaluated: Zorbax ODS, Zorbax TMS (DuPont), 6-μm, 250 × 4.6 mm I.D.; Spherisorb ODS (Spectra-Physics, Santa Clara, CA, U.S.A.), 10-μm, 250 × 2.1 mm I.D.

Methylene chloride extracts were concentrated in a Buchler flash evaporator (Buchler, Fort Lee, NJ, U.S.A.) connected to the house vacuum (ca. 20 in. Hg) and a water bath maintained at ca. 30°C. Retortable and boil-in-bag pouches were sealed with a Sentinel Model 12AS heat sealer (Packaging Industries, Hyannis, MA, U.S.A.).

Reagents and materials

The acetonitrile used in the mobile phase was HPLC grade (Fisher, Pittsburgh, PA, U.S.A.), and the mono- and disodium phosphate buffers were Fisher reagent grade. Water was prepared by passing the "house" distilled water through a Millipore (Bedford, MA, U.S.A.) Milli-Q purification system. Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) distilled-in-glass, pesticide grade, methylene chloride was used for the sample concentration procedures. The 2,4- and 2,6-TDA, 98% purity, were obtained from Aldrich (Milwaukee, WI, U.S.A.) and used as received.

The retortable pouches used in the extraction studies were obtained from four different manufacturers. The boil-in-bag pouches were all purchased locally.

Standard TDA solutions

Standard solutions of 2,4- and 2,6-TDA were prepared by dissolving known weights of TDA in known volumes of solvent. Typically a stock TDA solution in water was prepared at the parts-per-million (ppm) (milligrams per liter) level, and aliquots were taken and diluted to the desired ppb levels with aqueous pH 7.4 buffer having the following composition: 0.01 M HCl, 0.01 M NaOH, 0.001 M NaH$_2$PO$_4$, 0.003 M Na$_2$HPO$_4$. Both the stock (ppm-level) and the working (ppb-level) solutions were stable for periods of several weeks.

Boil-in-bag and retortable pouch extraction procedure

The aqueous boil-in-bag extracts were obtained by filling the bags with Milli-Q purified water (2.5 ml/in.^{2}) and heat-sealing them (25 p.s.i., 350°F, 2 sec), immersing the bags in a boiling water bath (ca. 212°F) for 2 h, and then transferring the contents while hot to suitable glass storage containers. Aqueous retortable pouch extracts were similarly obtained by filling the pouches with Milli-Q water (4 ml/in.^{2}), heat-sealing (25 p.s.i., 350°F, 2 sec), and retorting for 2 h (250°F, 15 p.s.i.). The pouches were allowed to cool and then were stored unopened at ambient temperature until analyzed.

Sample clean-up/concentration procedure

Before LC analysis, the aqueous sample extracts were "washed" and concentrated by the following methylene chloride extraction procedure. A 250-ml portion of aqueous sample extract was transferred to a 500-ml separatory funnel. A 1-ml portion of 0.4 M HCl was added and the mixture was shaken with 100 ml of methylene chloride. The methylene chloride layer, which contained the acidic and neutral extractable components, was drained off and discarded. The aqueous extract was then made basic by the addition of 1.5 ml of 0.4 M NaOH, followed by extraction with two 150-ml portions of methylene chloride. The methylene chloride extracts, which totaled 300 ml, were combined in a 500-ml round-bottom flask. A 4-ml portion of 0.01 M HCl was added as a holding solvent and the methylene chloride was removed under vacuum (ca. 20 in. Hg) at 30°C. The 4 ml of sample concentrate was transferred to a 5-ml volumetric flask with a disposable pipet and neutralized by dilution to volume with a solution having the following composition: 0.04 M NaOH, 0.005 M NaH$_2$PO$_4$, 0.015 M Na$_2$HPO$_4$.

RESULTS AND DISCUSSION

Analysis of basic solutes, such as 2,4-TDA, by reversed-phase (RP) LC often is complicated by adsorptive interactions with "uncapped" silanol groups present in the silica-based packing material. These adsorptive interactions contribute to chromatographic peak tailing and cause loss of resolution and sensitivity.

Ion-suppression and ion-pairing are two techniques used in RPLC to minimize the detrimental effects of these interactions[10-12]. The overall mechanisms involved in these two techniques are not fully understood, but both have been used to dramatically improve the chromatography of ionizable solutes.

In ion-suppression HPLC, buffers are added to the mobile phase to obtain a pH which minimizes ionization of the solute. Conversely, in ion-pairing HPLC the

mobile phase pH is adjusted to maximize ionization, and a counter-ion such as an alkyl sulfonic acid is added, which forms a "non-ionic, neutral pair". In both cases, the desired result is attainment of a "neutral" solute, one which is less attracted to the residual silanol groups.

We evaluated both of these techniques for the trace analysis of aqueous solutions of 2,4- and 2,6-TDA. Ion-suppression HPLC produced sharper, more symmetrical peaks than ion-pairing HPLC, enabling greater sensitivity and resolution of TDA. Therefore, ion-suppression HPLC was the technique selected for this work.

Ion-suppression HPLC

We achieved ion-suppression of TDA by buffering the acetonitrile–water mobile phase with mono- and dibasic sodium phosphate. The effect of mobile-phase pH on the HPLC determination of TDA was studied over the pH range 4.5–8.5. For a mobile-phase pH range of *ca.* 6.5 to 8.5, good peak symmetry and stable retention volumes were observed (Fig. 1A). When the mobile-phase pH was lower than *ca.* 6.5, chromatographic peak broadening occurred, resulting in diminished peak heights and a significant reduction in resolving power (Fig. 1B).
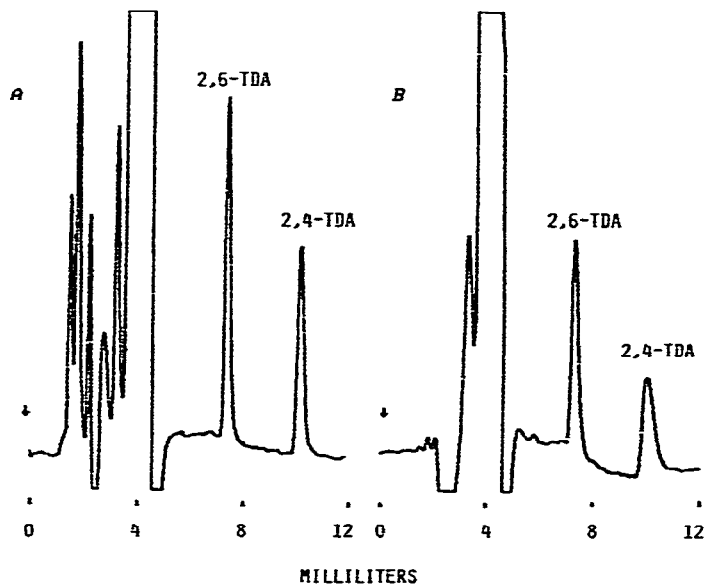

MILLILITERS

Fig. 1. Mobile-phase pH effect. Conditions: 500-μl injection volume, 1 ml/min, 40°C, 25 ppb of 2,4- and 2,6-TDA in pH 7.4 phosphate buffer, UV detector at 254 nm, 0.002 a.u.f.s. Mobile phase: (A) 10% acetonitrile/pH 7.4; (B) 10% acetonitrile/pH 5.0.

Consequently, we selected a mobile-phase pH of 7.4 (0.001 M NaH$_2$PO$_4$, 0.003 M Na$_2$HPO$_4$) to achieve the ion-suppression of TDA while staying within the pH restrictions of the silica-based column packing material. As a further precaution, we minimized any pH effect on the packing material by flushing the column nightly with 20 ml of water followed by 20 ml of acetonitrile. Columns treated in this manner exhibit useful lifetimes of 5–6 months.

The dependence of TDA retention volume on the acetonitrile concentration in

the pH 7.4 phosphate buffer was also studied (Table I). As would be expected for a C_8 reversed-phase column, the TDA retention volume increased as the acetonitrile concentration was decreased, particularly at acetonitrile concentrations lower than 8 %. Low acetonitrile concentrations also resulted in poor column efficiency, as evidenced by the increase in the height equivalent to a theoretical plate (HETP).

TABLE I

VARIATION IN RETENTION VOLUME OF TDA WITH PERCENT ACETONITRILE IN MOBILE PHASE

Conditions: Ultrasphere-octyl, 250 × 4.6 mm I.D. column, 1 ml/min, 40°C, 254 nm, 0.002 a.u.f.s., 500-μl injection of 25 ppb each of 2,4- and 2,6-TDA.

Acetonitrile (%)*	Retention volume (ml)		Peak height (mm)		HETP · 10^{-2} (mm)	
	2,6-TDA	2,4-TDA	2,6-TDA	2,4-TDA	2,6-TDA	2,4-TDA
20	5.1	5.9	150	105	1.5	1.3
15	5.8	7.2	120	75	1.6	1.5
10	7.0	9.8	80	50	1.9	1.7
8	8.0	11.8	70	40	2.1	1.7
5	10.0	16.0	50	25	2.7	1.9

* Percent acetonitrile in pH 7.4 phosphate buffer.

We found that 10 % acetonitrile in pH 7.4 phosphate buffer was the mobile phase best suited for the samples of interest in this study. Consistent retention volumes and adequate resolution from surrounding peaks were observed for most samples examined. With this mobile phase, 2,6- and 2,4-TDA retention volumes were *ca.* 6.8 and 9.3 ml, respectively; the corresponding relative standard deviations were ±0.5 and ±0.4 %.

Alternative reversed-phase columns

In addition to the Ultrasphere-octyl column, three other reversed-phase columns were evaluated for the HPLC determination of TDA. In these tests the Zorbax ODS and Ultrasphere C_8 columns produced very similar separations. The main difference between them was that the Zorbax ODS column showed slightly more retention of both TDA and the late-eluting components present in most samples analyzed. The second column, a Spherisorb ODS, exhibited limited suitability in actual sample analysis for TDA. Because of its larger particle diameter (10 μm) and smaller column inner diameter (2.1 mm) the number of theoretical plates obtained for this column was insufficient to separate the TDA chromatographic peak from the others surrounding it. With the third column tested, a Zorbax TMS, we were also unable to resolve TDA from interferences present in most sample extracts.

In general, the best separation of 2,4- and 2,6-TDA in aqueous extracts was achieved on the octyl, 5 μm, 250 × 4.6 mm I.D. column. A C_{18} reversed-phase column (5 μm, 250 × 4.6 mm I.D.) would probably be a suitable substitute; however, a slightly longer analysis turnaround time and a marginally broader 2,4-TDA peak might be experienced.

Injection volume

As stated above, TDA was strongly retained on the reversed-phase column when very low acetonitrile concentrations were used in the mobile phase. As a result, it was possible to obtain a "concentration effect" upon injection when distilled water or an aqueous buffer was used as the sample solvent; *i.e.*, TDA partitioned into the stationary phase at the very head of the column and did not begin to elute down the column until the sample solvent was replaced with the mobile phase containing acetonitrile. This selection of a sample solvent which was "weaker" than the mobile phase permitted an injection volume of 500 μl, rather than the typical injection volume of 10–50 μl. Thus sensitivity to TDA was maximized with minimal loss of column efficiency (Table II).

TABLE II

VARIATION IN TDA PEAK VOLUME WITH INJECTION VOLUME

Conditions: 10% acetonitrile/pH 7.4, 1 ml/min, 254 nm, 40°C, Ultrasphere-octyl column. Samples: 100 ppb 2,6- and 2,4-TDA in pH 7.4 phosphate buffer.

Injection volume (μl)	Peak volume* (μl)		Theoretical plates** per meter	
	2,4-TDA	2,6-TDA	2,4-TDA	2,6-TDA
20	295	195	62,000	70,000
50	300	205	61,000	66,000
200	315	220	57,000	59,000
500	345	250	51,000	52,000
1000	380	315	47,000	37,000

* Peak volume determined at base by tangent skimming.
** Theoretical plates/meter = [16 (ret. vol./peak vol.)^2] × 4.

This concentration effect is clearly seen in Table II where chromatographic peak volumes are compared with injection volumes. With a 20-μl injection volume, 2,4-TDA eluted *ca.* 9.2 ml after injection with a peak volume that measured *ca.* 295 μl. (Peak volumes were measured at the base by tangent skimming.) With a 500-μl injection volume, 2,4-TDA eluted *ca.* 9.7 ml after injection but the peak volume measured only *ca.* 345 μl, which was *ca.* 30% less than the original injection volume of 500 μl. The net result of this concentration effect was that the number of theoretical plates obtained with the 500-μl injection was roughly 80% of the number obtained with the 20-μl injection.

For this concentration effect to occur, it is imperative that the pH of the sample injected be slightly basic to prevent ionization of TDA; otherwise, much broader chromatographic peaks similar to those observed in Fig. 1B are produced. Therefore, the samples, like the mobile phase, were also buffered to pH 7.4 with mono- and dibasic sodium phosphate.

The routine use of this unusually large injection volume of 500 μl did pose one problem. Both the samples and the standards inevitably contained trace amounts of very fine particulate matter which were not removed by filtration before injection. A

variety of filters were examined, and all either partially retained TDA and/or gave rise
to numerous interfering peaks. Consequently, all injected samples and standards were
unfiltered and the particulate matter was allowed to accumulate on the column inlet
frit (5-μm pore diameter). As a result, the column inlet frit required replacement
about once a week. Replacement of the column inlet frit was indicated, not by an
increase in the column back-pressure, but by tailing of the chromatographic peaks.

We found that we could further improve chromatographic peak symmetry by
removing and repacking the first 2–3 mm of column packing material about once
every 2–3 weeks. Evidently some of the particulate matter was small enough to pass
through the inlet frit and accumulated on the head of the column.

Detector high-frequency noise

Operation of the UV detector at its maximum sensitivity of 0.002 a.u.f.s. yield-
ed a 20% deflection for a 500-μl injection of 20 ppb 2,4-TDA. However, con-
siderable high-frequency noise occurred at this sensitivity. We effectively removed
this high-frequency noise (Figs. 2A and 2B) by connecting the detector output to an
electronic noise filter, operated at a frequency cutoff of 0.02 Hz. The electronic filter
was also capable of amplifying the detector signal, and a usable baseline was obtained
with an amplification factor of 2. Under these conditions (0.002 a.u.f.s., amplification
× 2, frequency cutoff 0.02 Hz) a deflection of *ca.* 5% was recorded for a 500-μl injec-
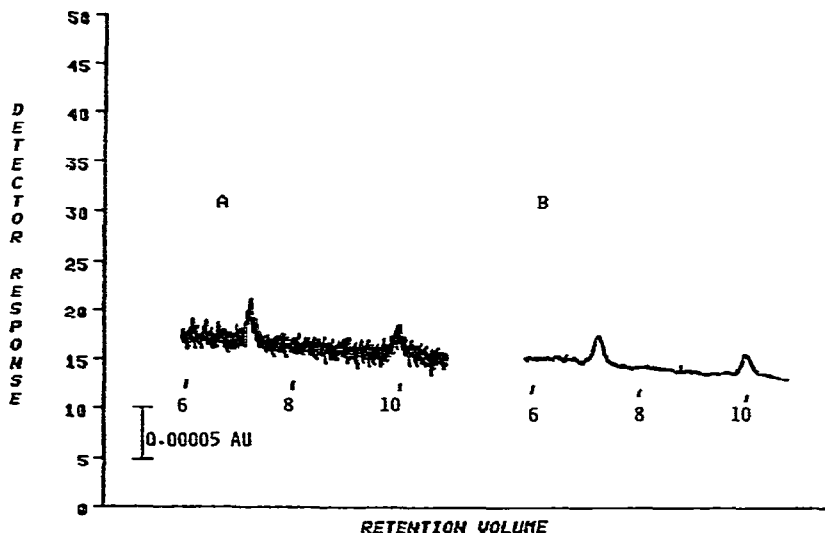tion of 2 ppb 2,4-TDA (Fig. 2B).

Fig. 2. Effects of detector high-frequency noise filtering. Conditions: 500-μl injection volume, 2 ppb of
2,6- and 2,4-TDA, 10% acetonitrile/pH 7.4, 1 ml/min, 0.002 a.u.f.s., amplification ×2. (A) No high-
frequency noise filtering; (B) 0.02-Hz cutoff frequency.

External standard calibration

TDA was quantitated by the external calibration technique; for calibration,
500-μl portions of standard solutions were injected for a series that contained 2,4- and
2,6-TDA in a 2–50 ppb concentration range. The peak maxima were recorded at each

concentration, and a linear regression was calculated. Fig. 3 shows a typical calibration plot having linear coefficients ($y = m\,x$) of better than 0.988 for 2,6- and 2,4-TDA with relative standard errors of ± 1 and $\pm 1.7\%$, respectively.

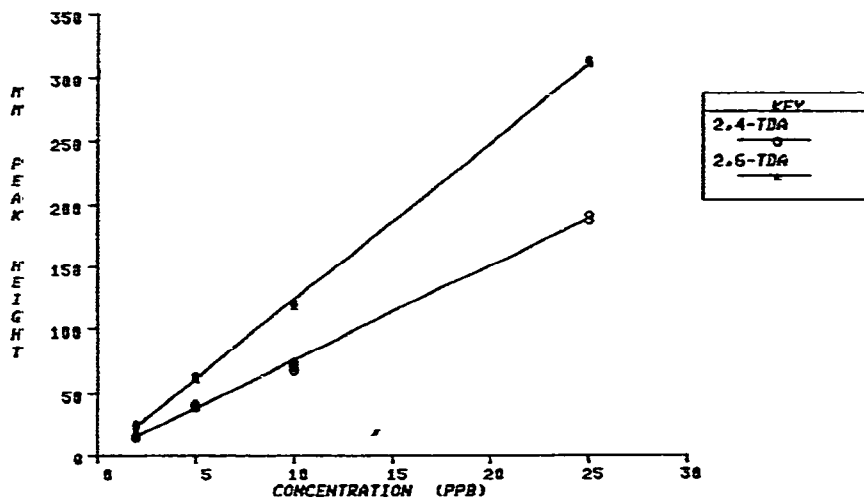2,4- AND 2,6-TDA LINEAR CALIBRATION

Fig. 3. 2,4- and 2,6-TDA linear calibration plots. LC conditions same as Fig. 2B.

Sample preparation

Aqueous extracts from a variety of boil-in-bag and retortable pouches were analyzed for 2,4- and 2,6-TDA under the LC conditions outlined above. We obtained the extracts by filling the boil-in-bag and retortable pouches with water (2.5–4 ml/in.²) and boiling (212°F) or retorting (250°F) for a specified length of time. Preliminary LC analyses of some of the extracts indicated that, if TDA was present, the concentrations were below the LC quantitation limit (2 ppb) and sample concentration would be required. In addition to any possible 2,4- and 2,6-TDA, numerous other components, presumably from both the plastic films and the laminating adhesives, were extracted under these conditions. Therefore a "sample clean-up" was also needed to remove these potentially interfering and late-eluting components.

Methylene chloride proved to be the ideal solvent for this clean-up and TDA extraction since partitioning of TDA between methylene chloride and water is very pH-dependent. Thus, when the samples (250 ml) were acidified (1 ml of 0.4 N HCl) as the first step, most of the interfering and late-eluting components were selectively extracted into the methylene chloride layer (100 ml) and all of the TDA was left behind. After next adding sodium hydroxide to make the sample basic (1.5 ml of 0.4 N NaOH), we achieved an almost quantitative extraction of TDA by re-extracting with two 150-ml portions of methylene chloride.

The TDA was concentrated by removal of the methylene chloride under vacuum (20 in. Hg) at 30°C. Evaporating the methylene chloride directly to dryness and subsequently dissolving the TDA in 5 ml of water gave low and inconsistent

recoveries. Adding 5 ml of water to the methylene chloride before evaporation improved the precision, but TDA recovery was only *ca.* 65%. Acidifying the 5 ml of water with HCl increased TDA recovery to *ca.* 90%.

Because it was necessary to neutralize the final concentration before injection into the LC, we used a 4-ml volume of 0.01 M HCl as the "holding solvent." After the methylene chloride was removed under vacuum, the 4 ml of 0.01 M HCl was quantitatively transferred to a 5-ml volumetric flask. A 1-ml portion of a 0.04 M NaOH solution was added, and the resulting solution was diluted to volume with water. The 0.04 M NaOH solution also consisted of 0.005 M NaH$_2$PO$_4$ and 0.015 M Na$_2$HPO$_4$, which helped to buffer the sample to the desired pH. The final 5-ml volume represented a 50-fold concentration of the TDA originally present in the 250-ml aqueous sample extract. Using less than 4 ml of 0.01 M HCl as the holding solvent to achieve a greater than 50-fold concentration gave poor TDA recoveries.

TABLE III

RECOVERY OF TDA WITH USE OF METHYLENE CHLORIDE EXTRACTION PROCEDURE

Solution analyzed	Added (ppb)	Found* (ppb)	Average recovery (%)
		2,6-TDA	
Standard	0.05	0.047, 0.052, 0.050	99
	0.10	0.092, 0.085, 0.085	87
	0.25	0.238, 0.233, 0.225	93
	0.50	0.435, 0.450, 0.435	88
	2.0	1.68, 1.73, 1.78	87
			—
Average ± SD			91 ± 3.5
Retortable pouch extract	0.00	1.62, 1.56, 1.54 (1.57)**	
	2.00	3.47, 3.62, 3.59 (3.64)	92
Boil-in-bag extract	0.00	0.09, 0.09, 0.09 (0.09)	
	0.20	0.25, 0.26, 0.26 (0.26)	85
		2,4-TDA	
Standard	0.05	0.041, 0.044, 0.049	89
	0.10	0.078, 0.081, 0.089	83
	0.20	0.190, 0.178, 0.178	91
	0.50	0.455, 0.455, 0.450	90
	1.0	0.88, 0.93, 0.92	91
			—
Average ± SD			88 ± 4.5
Retortable pouch extract	0.00	1.88, 1.86, 1.78 (1.84)	
	2.00	3.71, 3.62, 3.59 (3.64)	90
Boil-in-bag extract	0.00	0.06, 0.07, 0.06 (0.06)	
	0.20	0.22, 0.23, 0.23 (0.23)	84

* As determined by LC analysis.
** Average value is given in parentheses.

Precision and recovery

We determined the overall precision of the method and the anticipated levels of recovery by performing the methylene chloride concentration procedure, followed by LC analysis, with a series of aqueous standards. Triplicate, 250-ml portions of six standards, containing both 2,4-TDA and 2,6-TDA at concentrations ranging from 50 parts per trillion (ppt) (nanograms per liter) to 2 ppb, were concentrated to 5 ml and then quantitated by LC analysis. The results, presented in Table III, show average recoveries of 88% for 2,4-TDA and 91% for 2,6-TDA with relative standard deviations of ±4.5% and ±3.5%, respectively. Similar levels of precision and recovery were also found for spiked composites of retortable pouch and boil-in-bag extracts (Table III).

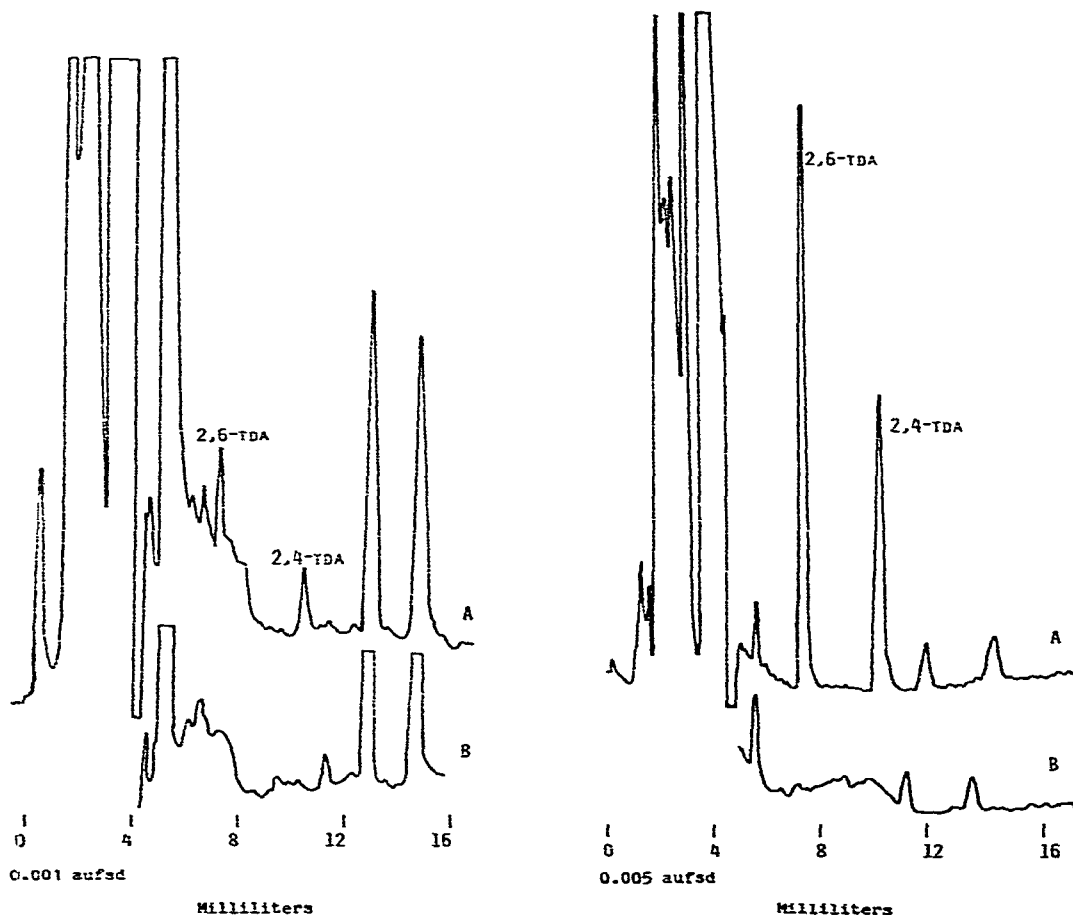Fig. 4A shows a chromatogram for a 500-μl injection of a 50 ppt aqueous

Fig. 4. (A) 500-μl injection of an aqueous 50 ppt 2,4- and 2,6-TDA standard carried through the methylene chloride 50-fold concentration procedure. (B) Distilled water blank carried through methylene chloride procedure. LC conditions same as Fig. 2B.

Fig. 5. (A) 500-μl injection of an aqueous 2 ppb 2,4- and 2,6-TDA standard carried through the methylene chloride 50-fold concentration procedure. (B) Distilled water blank carried through methylene chloride procedure. LC conditions same as Fig. 2B.

standard carried through the methylene chloride concentration procedure; the original aqueous sample extract was 250 ml, and the 500 μl injected was taken from the final 5-ml concentrate. We believe that 50 ppt is the lower limit of reliable quantitation. The calculated concentrations of 2,6- and 2,4-TDA for these peaks are 2.4 and 2.3 ppb, respectively, and indicate corresponding recoveries of 96 and 92%. Fig. 4B shows the chromatogram for a 500-μl injection of a "reagent blank", 250 ml of distilled water carried through the methylene chloride procedure, demonstrating the lack of any significant interferences even at a maximum sensitivity of 0.001 a.u.f.s. Fig. 5A and 5B show chromatograms for 500-μl injections of a 2-ppb aqueous standard of 2,4- and 2,6-TDA and a distilled water blank carried through the methylene chloride 50-fold concentration procedure.

Retortable pouch and boil-in-bag extracts

A variety of retortable pouch and boil-in-bag extracts were analyzed for apparent migration of 2,4- and/or 2,6-TDA. The extracts were obtained according to the conditions described in the Experimental section by extracting with distilled water at either 250 or 212°F for 2 h. Each aqueous extract was concentrated 50-fold by the methylene chloride extraction procedure followed by LC analysis. Apparent levels of 2,4- and 2,6-TDA migration measured in these extracts are listed in Table IV. Typical LC chromatograms for a boil-in-bag and a retortable pouch extract are represented in Figs. 6A and 6B, respectively. The apparent levels of 2,4- and 2,6-TDA are 1.8 and 1.6 ppb, respectively, for the retortable pouch extract and 0.1 and 0.2 ppb, respectively, for the boil-in-bag extract.

CONCLUSIONS

Ion-suppression was found to be a useful technique for the HPLC determi-

TABLE IV

APPARENT 2,4- AND 2,6-TDA MIGRATION FROM RETORTABLE POUCH AND BOIL-IN-BAG EXTRACTS

Manufacturer	*Apparent migration (ppb)*	
	2,4-TDA	*2,6-TDA*
	*Retortable pouch**	
A	5.0	5.0
B	2.0	4.0
C	1.5	2.5
D.	3.0	2.5
	*Boil-in-bag***	
E	0.05	0.08
F	0.4	0.6
G	ND***	ND***
H	0.7	0.7
I	0.1	0.2

* Retorted 2 h at 250°F (4 ml H_2O/in.^{2}).
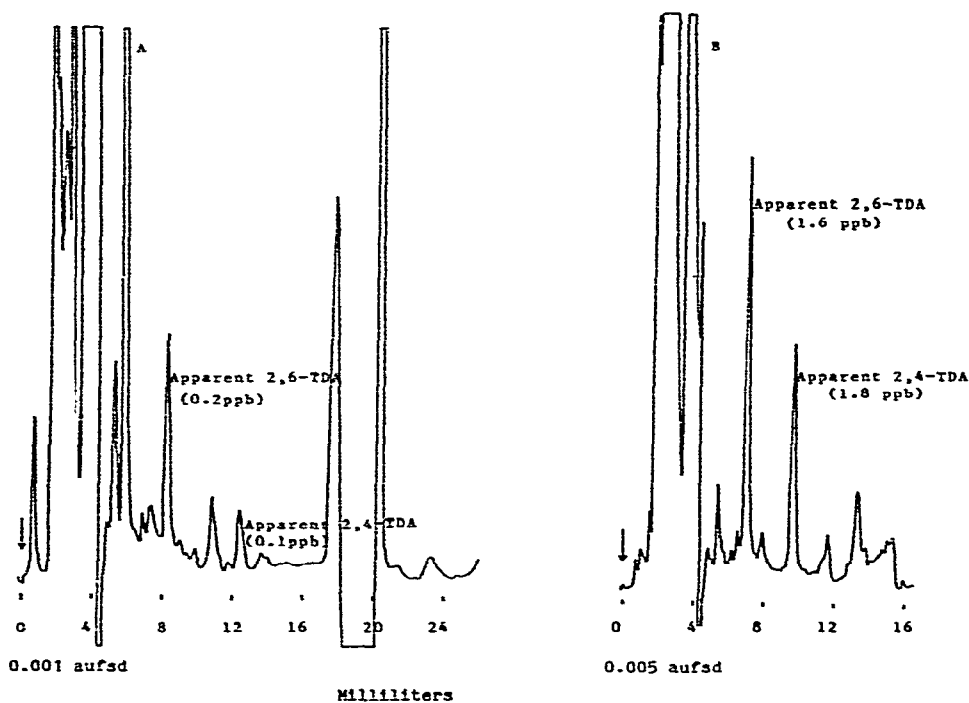** Boiled 2 h at 212°F (2.5 ml H_2O/in.^{2}).
*** ND, less than 40 ppt.

Fig. 6. (A) 500-μl injection of a boil-in-bag extract carried through the methylene chloride concentration procedure. (B) 500-μl injection of a retortable pouch extract carried through the methylene chloride concentration procedure. LC conditions same as Fig. 2B.

nation of 2,4- and 2,6-TDA in aqueous extracts. Quantitation of TDA at the ppt level was made possible by the exceptionally large injection volume of 500 μl. Apparent TDA concentrations ranging from 50 ppt to 5 ppb were measured in a variety of retorted pouch and boil-in-bag extracts. The data were compared with results obtained with a nitrogen-selective gas chromatographic procedure[13]. Analyses by gas chromatography–mass spectrometry afforded data consistent with the presence of TDA in the samples.

REFERENCES

1 E. K. Weisburger, A. B. Russfield, F. Homburger, J. H. Weisburger, E. Boger, C. G. VanDongen and K. C. Chu, J. Environ. Pathol. Toxicol., 2 (1978) 325.
2 International Agency for Research on Cancer, IARC Monographs on the Evaluations of the Cancer Risk of Chemicals to Man, Lyon, France, June 7–14, 1977, World Health Organization, Switzerland, 1978, Vol. 16, p. 83.
3 N. Ito, Y. Hiasa, Y. Konishi and M. Marugami, Cancer Res., 29 (1969) 1137.
4 B. Olufsen, J. Chromatogr., 179 (1979) 97.
5 C. E. Boufford, J. Gas Chromatogr., 6 (1968) 438.
6 F. Willeboordse, Q. Quick and E. T. Bishop, Anal. Chem., 40 (1968) 1455.
7 L. E. Brydia and F. Willeboordse, Anal. Chem., 40 (1968) 111.
8 P. D. Unger and M. A. Friedman, J. Chromatogr., 174 (1979) 379.
9 K. J. Williams, A. Li Wan Po and W. J. Irwin, J. Chromatogr., 194 (1980) 217.
10 B. A. Bidlingmeyer, J. Chromatogr. Sci., 18 (1980) 525.
11 R. Gloor and E. L. Johnson, J. Chromatogr. Sci., 15 (1977) 413.
12 R. G. Achari and J. T. Jacob, J. Liq. Chromatogr., 3 (1980) 81.
13 R. C. Snyder and C. V. Breder, in preparation.