Determination of Zinc Dialkyldithiocarbamates in Latex Condoms

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Abstract

A simple high-performance liquid chromatographic (HPLC) assay is developed for measuring zinc dialkyldithiocarbamate (DTC) levels in latex condoms. After extraction of 14 different brands of latex condoms in acetonitrile, aliquots of the extracts are subjected to a preliminary screening assay by treatment with cobalt chloride and measurement of UV absorption at 320 nm, which results in the identification of 6 DTC-containing samples. Prior to analysis by HPLC, zinc dimethyldithiocarbamate (ZDMC) or zinc diethyldithiocarbamate (ZDEC) is added to the extracts in order to block transmetalation reactions with the analytes of interest. A reversed-phase C_{18} column, with gradient elution and UV detection at 260 nm, is used to measure the zinc DTCs. The limits of detection for ZDEC and zinc dibutyldithiocarbamate (ZDBC) are 5 and 10 µg/mL. Levels of ZDBC and ZDEC range from not detectable to 3.31 and 1.79 mg/condom, respectively. Total protein and latex allergenic protein levels are determined and range from 98 to 776 and 0.01 to 14.04 µg/unit, respectively, but are not related to the level of ZDBC or ZDEC. This methodology provides both screening and specific tools for the determination of unstable zinc DTC complexes in latex products.

Introduction

N,N-dialkyldithiocarbamates (DTCs) are often used as vulcanization accelerators in latex products. Zinc DTC accelerators are added either directly or are formed in situ during the vulcanization process via a reaction between a thiuram and zinc oxide (1,2). Zinc DTCs have been detected in leachates from medical devices such as gloves, and studies have shown these complexes to be causative agents of allergic contact dermatitis (3–7). Consequently, a simple selective method is required to monitor zinc DTC levels in latex extracts.

A number of methods have been reported for the analysis of metal DTCs, with liquid chromatography (LC) being the most commonly used (3–20). However, the determination of zinc DTC complexes does not appear to be straightforward (15,16,18–20). Metal exchange reactions between these complexes and nickel from the stainless steel components of the chromatographic system were reported, which precluded their direct analysis. Attempts to minimize these exchange reactions with the use of a metal-free column or the addition of an ion-pairing reagent such as ethylenediaminetetraacetic acid or DTC ligand were unsuccessful.

Two methods have been reported that facilitated the analysis of zinc DTC levels in rubber extracts, but each has its disadvantages. The first and most commonly used method involves precolumn derivatization of zinc DTC compounds with cobalt, resulting in formation of stable Co(III) DTC complexes suitable for reversed-phase high-performance liquid chromatography (HPLC) (3–5,18). However, this method is severely limited when more than one type of zinc DTC accelerator is present in samples, as multiple mixed ligand cobalt(III) DTCs are formed. The method also cannot distinguish free DTC from DTC incorporated in zinc DTC complexes. These problems were overcome by the method of Mathieu et al., which minimized exposure of the sample to metal components by using polyether ether ketone (PEEK)-lined chromatographic components coupled with saturation of the system with zinc ions (16). The practicality of this method is limited by the cost associated with outfitting an HPLC with PEEK-lined componentry.

In this paper we describe a simple preliminary screening method for quantitation of total DTC from latex utilizing the lability of these complexes and the formation of a cobalt(III) DTC chromophore. Samples that tested positive by this screening method were then analyzed directly by HPLC, and the zinc DTC complexes were identified and quantitated. Chemical speciation is important because of the wide range of reported toxicological potency between zinc DTC species (21,22). Latex condoms from a variety of manufacturers were analyzed using these procedures. Results are presented showing that no precolumn derivatization or special HPLC modifications were required in order to successfully analyze zinc DTC compounds. Problems associated with transmetalation reactions were overcome by the addition of an alternate zinc DTC complex that served as a suitable protecting agent.

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Experimental

Materials and reagents

Zinc diethyldithiocarbamate (ZDEC), zinc dimethyldithiocarbamate (ZDMC), cobalt chloride hexahydrate, sodium diethyldithiocarbamate, and HPLC-grade acetonitrile (ACN) were obtained from Aldrich (Milwaukee, WI). Zinc dibutyldithiocarbamate (ZDBC) and nickel dibutyldithiocarbamate (NDBC) were acquired from Chem Service (West Chester, PA). Zinc pentamethylene dithiocarbamate was obtained from Pfaltz & Bauer (Waterbury, CT). Distilled water used throughout was purified by a Millipore Milli Q system (Molshiem, France). All solvents used for HPLC were filtered (0.22-µm membrane) and degassed by sonication under vacuum for 10 min at ambient temperature. Cobalt chloride was prepared as a 420-mmol/L solution in water. A 500-µg/mL ZDMC solution was prepared in ACN. ZDEC and ZDBC stock solutions (1000 µg/mL) were prepared each time in ACN. Working standard solutions used for UV detection were prepared by the appropriate dilutions in ACN to yield final concentrations of 31.3, 62.5, 125, 250, and 500 µg/mL. Fourteen brands of condoms from a range of different manufacturers were purchased commercially.

Instrumentation

Absorbance measurements of cobalt(III) DTC complexes were carried out on a Beckman DU 650 (Fullerton, CA) spectrophotometer at 320 nm.

HPLC analysis was performed on a Shimadzu (Kyoto, Japan) system consisting of two LC-600 pumps, an SIL-10AD autoinjector, LPI-6B interface, and SPD-M10A diode array detector. An injection volume of 10 µL was used, and separations were performed on a 5-µm particle size, 250- × 4.6-mm Supelco Discovery C_{18} column (Bellefonte, PA) at a flow rate of 1 mL/min. An absorbance at 260 nm was used for the UV detection of zinc DTC complexes. The elution gradient profile started at 85% acetonitrile–water and was increased at a rate of 5%/min for 3 min before being held at 100% acetonitrile for 11 min.

Gas chromatography (GC)–mass spectrometry (MS) analyses were carried out using a Hewlett Packard 5890 series II (Palo Alto, CA) GC coupled to a Hewlett Packard 5972 series mass selective detector. The GC was equipped with a split/splitless injector operating at 250ºC in the splitless mode. For separation, an HP-5MS column (30-m × 0.25-mm i.d, 0.25-µm film thickness) (J&W Scientific, Folsom, CA) was used with helium carrier gas at a flow rate of 1 mL/min. The mass selective detector was maintained at 280ºC. After injections of 1 µL, an initial oven temperature of 50ºC was maintained for 2 min, then ramped to 310ºC at 10ºC/min, and held for 1 min. The MS was operated in electron impact mode with an ionization potential of 70 eV, and the spectra were obtained in full scan mode.

Determination of zinc DTCs in latex extracts

Each condom was weighed before being cut into approximately 1-cm strips and then extracted using 20 mL ACN for 17 h with agitation at room temperature. Preliminary screening involved treating aliquots (600 µL) of the extracts and standards with $CoCl_2$ (6 µL 420 mmol/L) and then agitating them for 30 min at ambient temperature. Samples (100 µL) were taken and diluted with ACN (1:20) prior to measuring their absorbance on a UV spectrophotometer.

Fresh 600-µL aliquots of positive testing extracts (> 31.3 µg/mL), along with standards, were taken and added to tubes containing 300 µg ZDMC (or ZDEC for analysis of ZDMC) (600-µL aliquots of 500 µg/mL taken down to dryness under a constant stream of nitrogen at 30ºC). The tubes were then shaken until ZDMC/ZDEC redissolved. Prior to analysis, samples were then filtered and the HPLC conditioned by two 50-µL injections of a saturated ZDMC solution (or ZDEC for ZDMC analysis) in ACN. All determinations were made in duplicate from two separate condoms from the same lot for each brand tested.

Assay validation

Accuracy, intra-, and interday precisions of the method were determined by analyzing five replicate standard samples in ACN at two different ZDEC and ZDBC concentrations (62.5 and 250 µg/mL) on three separate days. These quality control samples were prepared independently of the calibration standards. The intra- and interday precisions were calculated as the coefficients of variation. Accuracy was calculated by back calculation of the standards and comparison with the known spiked concentration and expressed as percent bias.

Determination of protein content in latex extracts

Each condom was weighed before being cut into approximately 1-cm strips then extracted using 20 mL phosphate buffered saline (PBS) (pH 7.4) for 21 h with agitation. Aliquots (15 mL) were filtered then dialyzed in H_2O at 4ºC for 18 h. The dialyzed samples were then lyophilized and diluted to 1 mL in PBS. Total protein content (20-µL aliquots) was determined using a modified Lowry method (25).

Allergen levels were determined by measuring the ability of samples to inhibit a fluoroenzyme immunoassay (FEIA) for natural rubber latex-specific human immunoglobin E (IgE) (CAP System, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). The specific IgE FEIA assay was performed according to the manufacturer's kit instruction. A commercially prepared nonammoniated latex (NAL) allergen extract (2 mg/mL) was purchased from Greer Laboratories (Renoir, NC) to set up an eight-point standard curve

Table I. Determined DTC Level in Latex Condom Extracts

	Preliminary assay*		HPLC assay		
Brand	mg/unit	µg/mg unit	mg/unit	µg/mg unit	Zinc DTC type
A	3.96	2.50	3.31	2.09	ZDBC
B†	2.71	1.67	1.28	0.79	ZDEC
C†	3.87	2.09	1.79	0.96	ZDEC
D†	2.99	0.99	2.11	0.70	ZDBC
E†	2.65	1.29	1.31	0.65	ZDBC
F†	3.83	2.22	3.12	1.81	ZDBC
G–N‡	ND§	ND	ND	ND	ND

* Reported as ZDBC equivalent.
† Brand contained lubricant.
‡ Includes both lubricated and nonlubricated brands.
§ ND = not detected.

to measure concentration of the latex allergen in the sample extracts by competitive binding (inhibition assay) with the NAL standards ranging from 0–100 µg/mL NAL. All standards, controls, and samples were run in duplicate. The kU/L allergen concentration for the NAL standards and sample extracts determined by the CAP immunoassay were entered into the KC4, v2.5 data reduction software program (Bio-Tek Instruments, Inc., Winooski, VT). The NAL allergen concentrations were extrapolated from a four-parameter logistic fit curve.

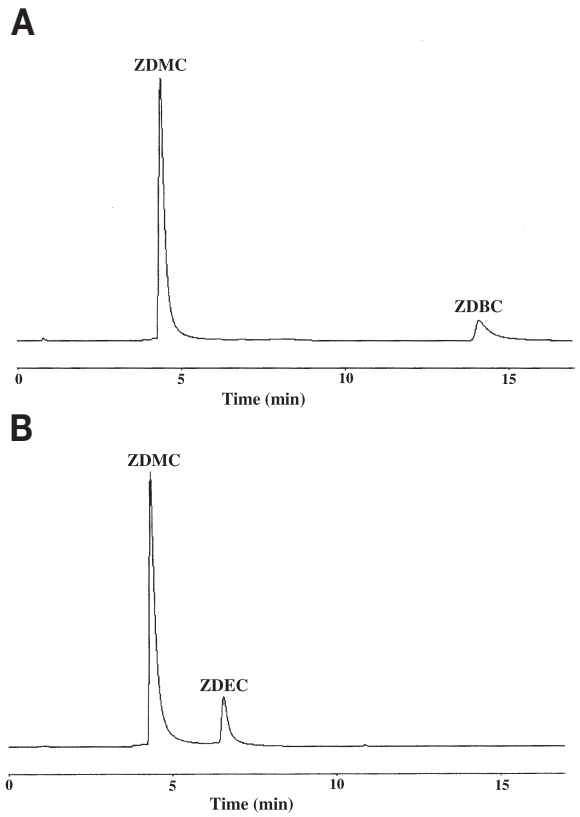

Figure 1. Chromatograms of condom extracts for zinc DTC complexes. Brand A (A) contained ZDBC and Brand C (B), ZDEC. ZDMC (300 µg) was added prior to analysis to block transmetalation reactions between extract zinc DTCs and metal ions leached from components of the HPLC system. DTCs were detected at 260 nm following elution from a C_{18} chromatographic column.

Results and Discussion

Zinc DTC levels in 14 different brands of condoms were determined using the following steps: (*i*) extraction of latex additives into acetonitrile, (*ii*) preliminary screening and estimation of total DTC content of extracts with cobalt chloride, and (*iii*) addition of a "protecting" zinc DTC complex prior to separation of zinc DTC complexes by reversed-phase chromatography with UV detection. The levels of zinc DTC detected in the latex extracts are given in Table I. Representative chromatograms of ZDEC and ZDBC from latex condom extracts are shown in Figure 1. Under the conditions described, retention times of 5.3, 7.4, and 14.5 min were observed for ZDMC, ZDEC, and ZDBC, respectively.

A quick and simple screening assay was developed to differentiate between samples containing DTCs and those that did not. Addition of cobalt chloride to zinc DTC-containing samples facilitated a transmetalation reaction, resulting in the formation of cobalt(III) dithiocarbamate complexes. The formation of green-colored solutions upon treatment of sample extracts treated with cobalt chloride proved to be a very good indicator of the presence of zinc DTCs. This is illustrated by the transmetalation of ZDEC and ZDBC standards, which both yielded green-colored solutions whose intensity faded to light green and then to blue as the concentration of standard decreased. The lowest concentration at which ZDEC and ZDBC could be detected visually was 62.5 µg/mL. Excellent linear plots from 1000 to 31.3 µg/mL were obtained when direct absorbance measurements at 320 nm were made and plotted against the concentration of ZDBC. Despite the omission of a chromatography step, this method provides a simple screening tool to evaluate whether samples contain DTCs. It provides a means to establish a rough estimate of the total DTC content in a sample. Unfortunately it does not identify the type of DTC or whether it is bound or exists as a free ligand. Quantitation of ZDEC from a ZDBC standard curve was also found to result in an approximately 12% overestimation of known levels. When analyzing large numbers of samples, this preliminary screening method should prove useful for identifying latex products with free DTCs.

Of the 14 brands tested, 6 tested positive for the presence of zinc DTCs (Table I). ZDEC was identified in two of these by GC–MS, whereas dibutylamine was detected in the other four. It is likely that dibutylamine is a thermal degradation product of ZDBC. The remaining eight samples showed no sign of any zinc DTCs or thermal degradation products. Further work also showed ZDMC and zinc pentamethylene dithiocarbamate could not be detected by GC–MS. It is apparent that the majority of zinc DTCs are not suitable for analysis via GC–MS under the conditions used. Accurate quantitation of ZDEC by GC–MS was precluded

Table II. Accuracy and Precision Data for the Determination of ZDEC and ZDBC in ACN

ZDEC				ZDBC			
Spiked concentration (µg/mL)	Back calculated concentration ± SD	%CV	%Bias*	Spiked concentration (µg/mL)	Back calculated concentration ± SD	%CV	%Bias
Intraday†	(*n* = 5)			Intraday	(*n* = 5)		
62.5	64.6 ± 3.4	5.2	3.4	62.5	64.1 ± 0.8	1.2	2.6
250	256.7 ± 3.6	1.4	2.7	250	241.3 ± 4.4	1.8	3.5
Interday	(*n* = 3)			Interday	(*n* = 3)		
62.5	59.1 ± 4.7	8.0	5.8	62.5	65.5 ± 4.6	7.0	4.6
250	251.8 ± 4.9	4.9	0.7	250	238.1 ± 5.5	2.3	5.0

* Bias = (observed concentration – nominal concentration)/nominal concentration.
† Intra- and interday precision is defined in the text.

because of complexities presumably arising from its thermal instability, resulting in poor linear standard curves and sensitivity.

In order to confirm the identity and determine levels of any zinc DTCs present in latex extracts, a suitable HPLC method was sought. Detection of zinc DTC complexes via HPLC is complicated by their labile nature. Previous reports (15,16,18–20) have shown a propensity for these complexes to undergo transmetalation reactions with metal ions leached from metal components of the chromatographic system, leading to the formation of multiple peaks or the absence of any peaks altogether. Determination of zinc DTC levels in latex extracts by conversion of labile zinc DTC complexes to nonlabile cobalt(III) dialkyldithiocarbamate complexes was initially attempted, but was found to be unsatisfactory because of poor peak shape, the presence of extraneous peaks, and poor linear standard curves observed for the prepared standards.

A new method was developed that allowed the direct determination of zinc DTC levels in extracts without the need for precolumn zinc DTC derivatization or the use of a PEEK-outfitted HPLC system. Problems associated with the instability of zinc DTC complexes with respect to transmetalation were overcome by the introduction of an additional zinc DTC complex. The addition of ZDMC to sample extracts and presaturation of the HPLC system with a saturated ZDMC solution successfully protected lower levels of ZDEC and ZDBC. The presence of ZDEC was confirmed in two samples, and ZDBC was detected in the remaining four. ZDMC presumably acts as a protecting agent by effectively complexing metal ions leached from the chromatographic system, thereby keeping the concentration to a minimum and limiting any detrimental effect on the compounds of interest. ZDEC was used as the protecting agent with an alternate set of fresh extracts to look for the presence of ZDMC in the latex condom extracts. ZDEC was found to effectively protect both ZDMC and ZDBC standards (31.3–500 µg/mL) from transmetal ation reactions with metal ions leached from HPLC components.

Table III. Protein Content of Latex Condom Extracts

	Lowry method		Quantitative ELISA*	
Brand	µg/unit	µg/g unit	µg/unit	µg/g unit
A	263	176	0.02	0.01
B	102	66	0.03	0.02
C	364	202	6.32	3.50
D	201	68	0.64	0.22
E	776	392	14.04	7.09
F	213	122	0.34	0.19
G	98	44	0.81	0.36
H	289	148	0.22	0.11
I	288	132	0.07	0.03
J	322	162	0.04	0.02
K	224	109	2.48	1.20
L	576	340	7.21	4.26
M	152	119	1.07	0.83
N	139	88	0.58	0.04

* ELISA = enzyme-linked immunosorbent assay.

None of the extracts were found to contain ZDMC, as a result, comprehensive validation studies on ZDMC were not performed. However, it was readily apparent that ZDMC sensitivity was very similar to that of ZDEC.

Results of the HPLC method validation studies are reported in Table II. Good accuracy and precision of the method were found. Intraday precision ranged from 1.4–5.2% for ZDEC and 1.2–1.8% for ZDBC, whereas interday precision ranged from approximately 2.3–8.0% in both complexes. The back calculated concentrations of both zinc DTC complexes were within 6% of the nominal value. Standard curves from 31.3 to 500 µg/mL ZDEC and ZDBC were linear and always gave correlation coefficients higher than 0.997, as determined by least-squares analysis. The limit of detection determined at a signal-to-noise ratio of 3 was 5 µg/mL ZDEC and 10 µg/mL ZDBC.

Zinc DTCs have been reported to induce contact hypersensitivity reactions in humans (23). Contact hypersensitivity is a cell-mediated, delayed-type reaction. De Jong et al. (21) screened the allergenic potential of various thiuram, dithiocarbamate, and benzothiazole latex additives using a modified mouse local lymph node assay. They reported a wide range of potency using this screening assay. ZDEC was found to be the most potent sensitizer, whereas ZDBC was the least potent and inactive in their screening assay. Tsuchiya et al. (22) also found differences between ZDEC and ZDBC potency in a variety of cytotoxicity tests. In light of the reported vast differences in potency between various zinc DTCs, chemical speciation is very important when measuring these in latex medical devices.

Total protein and latex allergenic protein levels were determined in all 14 brands of condoms tested (Table III). Protein ranged from 98 to 776 µg protein per condom. There was a good correlation between total and allergenic protein (r^2 = 0.791). There was no relationship between levels of protein or zinc DTC from these samples. This is not unexpected, as the proteins are naturally occurring plant-derived substances, whereas the zinc DTCs are added during latex processing.

The mechanisms and type of hypersensitivity reactions caused by zinc DTCs and latex proteins (IgE-mediated reaction) are different. However, the presence of high levels of specific zinc DTCs may potentially alter the allergenic potential/risk from the latex protein. Latex protein must cross the epidermis for it to be allergenic. Hayes et al. (24) reported that disruption of this barrier could facilitate the bioavailability of latex proteins. Irritation or contact dermatitis produced by zinc DTCs may potentially disrupt this barrier, however, their effect on subsequent immunological events involved in IgE-mediated allergies is not known.

Conclusion

In conclusion, simple, rapid, and reproducible screening and chemical speciation methods were developed for the determination of zinc DTC complexes in latex products. These methods were successfully used in the identification and quantitation of both ZDEC and ZDBC in latex condoms.

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