(6) M. Imai, *Eur. J. Pharmacol.*, **41**, 409 (1977).
(7) S. G. Karlander, R. Henning, and O. Lundvall, *Eur. J. Clin. Pharmacol.*, **6**, 220 (1973).
(8) A. C. Bollerup, B. Hesse, and B. Sigurd, *Acta Pharmacol. Toxicol.*, **34**, 305 (1974).
(9) K. L. Duchin and D. E. Hutcheon, *J. Pharmacol. Exp. Ther.*, **204**, 135 (1978).
(10) T. Higashio, Y. Abe, and K. Yamamoto, *J. Pharmacol. Exp. Ther.*, **207**, 212 (1978).
(11) U. B. Olsen and I. Ahnfelt-Rønne, *Acta Physiol. Scand.*, **97**, 251 (1976).
(12) U. B. Olsen, *Acta Pharmacol. Toxicol.*, **37**, 65 (1975).
(13) E. H. Østergaard, M. P. Magnussen, C. K. Nielsen, E. Eilertsen, and H. H. Frey, *Arzneim.-Forsch.*, **22**, 66 (1972).
(14) M. R. Cohen, E. Hinsch, R. Vergona, J. Ryan, S. J. Kolis, and M. A. Schwartz, *J. Pharmacol. Exp. Ther.*, **197**, 697 (1976).
(15) C. Brater, P. Chennavasin, J. M. Beck, and W. R. Fox, *Clin. Pharmacol. Ther.*, **27**, 421 (1980).
(16) J. Kaufman, R. Hamburger, J. Matheson, and W. Flamenbaum, *J. Clin. Pharmacol.*, **21**, 663 (1981).
(17) R. Pedrinelli, A. Magagna, F. Arzilli, P. Sassano, and A. Salvetti, *Clin. Pharmacol. Ther.*, **28**, 722 (1980).
(18) D. E. Smith, *J. Pharm. Sci.*, **71**, 520 (1982).
(19) J. G. Wagner, *J. Pharmacokinet. Biopharm.*, **5**, 161 (1977).
(20) K. Yamaoka, T. Nakagawa, and T. Uno, *J. Pharmacokinet. Biopharm.*, **6**, 165 (1978).
(21) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence Publications, Hamilton, Ill., 1975, pp. 38, 83, 349.
(22) J. B. Stokes, *J. Clin. Invest.*, **64**, 495 (1979).

ACKNOWLEDGMENTS

Supported in part by a Biomedical Research Support Grant from the College of Pharmacy and a Faculty Research Rackham Grant, The University of Michigan. During the course of this work H. S. H. Lau was supported as an NIH Predoctoral Scholar on NIH Training Grant GM 07767-04.

The authors thank Dr. J. L. Fox for the availability of his computer programs and expertise.

Contamination of Injectable Solutions with 2-Mercaptobenzothiazole Leached from Rubber Closures

JOHN C. REEPMEYER ˣ **and YVONNE H. JUHL**

Received June 18, 1982, from the *Food and Drug Administration, National Center for Drug Analysis, St. Louis, MO 63101.* Accepted for publication October 27, 1982.

Abstract □ An impurity, discovered in a sample of digoxin injectable solution commercially packaged in a syringe for single-dose delivery, was found to originate from the rubber closure of the syringe and was identified as 2-mercaptobenzothiazole, a common accelerator for rubber vulcanization. Several similarly packaged injectable solutions of a variety of drugs from various manufacturers were examined and over half contained 2-mercaptobenzothiazole. The compound was identified by UV spectrophotometry (including a pH-dependent shift in its absorbance maximum), by mass spectrometry, and by comparison with standard 2-mercaptobenzothiazole using silica gel and reverse-phase high-performance liquid chromatography (HPLC). The presence of this impurity in injectable solutions may have implications with regard to toxicity and may interfere with the assay of digoxin injectable solution by HPLC.

Keyphrases □ Injectable formulations—contamination by 2-mercaptobenzothiazole leached from rubber closures, single-dose syringes, syringe cartridges □ 2-Mercaptobenzothiazole—contaminant of injectable solutions, leached from rubber closures, single-dose syringes, syringe cartridges □ Drug packaging—injectable solutions, single-dose syringes, and syringe cartridges, contamination by 2-mercaptobenzothiazole leached from rubber closures

During the assay for digoxin in injectable solutions by reverse-phase high-performance liquid chromatography (HPLC) conducted according to the USP method (1), an impurity was discovered in a sample commercially packaged in a syringe for a single-dose delivery. The small variation in mobile phase compositions permitted by the method produced considerable differences in resolution of digoxin from its contaminant and differences in the digoxin assays. When the mobile phase composition was varied, a significant difference was observed between the change in retention time of digoxin and that of the impurity, which implied that the impurity was structurally unrelated to digoxin. The origin, identification, and significance of this impurity are discussed in this report.

EXPERIMENTAL

Reverse-Phase HPLC—For the analysis of digoxin injectable solutions, the HPLC system consisted of a liquid chromatograph[1], a variable-wavelength detector[2] set at 218 nm and 0.2 AUFS, a recorder–integrator[3] with a chart speed of 0.5 cm/min, and an automatic injector[4] set to inject 20 μl. A reverse-phase C18 column[5] and a mobile phase of 30% aqueous acetonitrile[6] were used; the flow rate was 2.0 ml/min. The digoxin injectable solution samples were used undiluted (0.25 mg/ml). To determine if the contaminant in the digoxin injectable solution was a cardiac glycoside related to digoxin, samples of digoxigenin mono- and bisdigitoxoside[7], digoxigenin[7], and diginatin[7] were chromatographed twice, with 26 and 30% acetonitrile as mobile phases, and were compared by retention time to the impurity.

Concomitant Use of HPLC and UV Spectrophotometry—To obtain a full UV spectrum of chromatographically pure compound, the column effluent was passed first through a detector[8], fixed at 254 nm and connected to a recorder[9] to produce a chromatogram and then through a 10-mm flow cell positioned in a rapid-scanning spectrophotometer[10] to produce the spectrum. As the mobile phase passed through the flow cell, UV spectra were recorded every 2 sec until the intensity of the signal reached a maximum, at which time the solvent flow from the column was diverted, locking the sample in the flow cell. This permitted repetitive scanning of the sample and produced a smooth spectrum of the com-

[1] Model 204 liquid chromatograph; Waters Associates, Millipore Corp., Milford, MA 01757.
[2] Model 450 variable-wavelength detector; Waters Associates.
[3] Data Module; Waters Associates.
[4] WISP 710B; Waters Associates.
[5] μBondapak C-18, 10-μm particle size, 300 mm (length) × 3.9 mm (i.d.); Waters Associates.
[6] For the chromatographic column used in this work, 30% acetonitrile was preferred over 26% acetonitrile (the concentration recommended by the USP) because elution time was shortened without chromatographic interference from related cardiac glycosides. With 30% acetonitrile, the system suitability requirements of the USP (1) were met.
[7] Burroughs Wellcome Co., Inc., Research Triangle Park, NC 27709.
[8] Model 440 absorbance detector; Waters Associates.
[9] Model 3390A Reporting Integrator; Hewlett-Packard Co., Palo Alto, CA 94304.
[10] Model 8450A UV/visible spectrophotometer; Hewlett-Packard Co.

0022-3549/83/1100-1302$01.00/0

pound even at extremely low concentrations[11]. In this manner, a full spectrum of any or all compounds in a chromatogram could be generated. Samples were injected manually[12].

The UV spectrum of the impurity was obtained in this manner by injecting the digoxin injectable solution. Small variations seen in the absorbance maximum of the impurity seemed to coincide with slight variations in the mobile phase composition. To determine if this variation in the absorbance maximum was due to small changes in pH, a fraction of eluant containing the impurity was collected from the column into a microcell and treated with small aliquots of 2 *N* NaOH, 1 *N* HCl, and pH 8 buffer solution. The absorbance maximum was measured at various pH values.

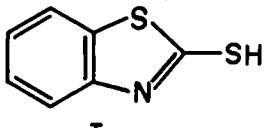
Extraction of Digoxin Injectable Solution—The contents of one 2-ml syringe of digoxin injectable solution was mixed with 2 ml of 1 *N* HCl and extracted with two 5-ml portions of chloroform. The aqueous acidic solution was made strongly basic with 2 *N* NaOH and extracted with two additional 5-ml portions of chloroform. A 400-μl aliquot from each of the four chloroform extracts was evaporated to dryness under a nitrogen stream; the residue from each was dissolved in 100 μl of 30% aqueous acetonitrile and analyzed by HPLC and UV spectroscopy using the method described above. Only the first chloroform extract contained the impurity. This solution was then extracted with 10 ml of 1 *N* NaOH. The aqueous solution was acidified with 2 *N* HCl to pH 1 and extracted with 5 ml of chloroform. The impurity was found only in this final chloroform extract.

Extraction of Container Parts—An empty digoxin injection container was separated into three parts: the rubber sheath which covered the needle, the glass barrel with its attached needle, and the rubber closure. Each part was submerged in a mixture of propylene glycol–ethanol–water (40:10:50), the solvent mixture used for the injection medium, and heated in an oil bath maintained at 60° for 2 weeks[13]. Extracts were examined for the presence of the impurity by HPLC.

Mass Spectroscopy—Since larger quantities of contaminant were available from the rubber parts of the injection container than from the injectable solution, the rubber sheath was selected as a source of a sample of the compound for mass spectroscopy. To avoid contamination of the compound with propylene glycol, one of the three solvents in the mixture originally used to extract the compound from the rubber sheath, other extraction solvents were sought: dilute NaOH solution was found to be suitable.

A 4-mm section of the rubber sheath was placed in 2 ml of 1 *N* NaOH in a tightly capped reaction vial and heated for 3 days in an oil bath maintained at 65°. The aqueous solution was extracted with two 3-ml portions of dichloromethane, acidified with 2 *N* HCl, and extracted with two 2.5-ml portions of dichloromethane. The latter two extracts were combined, and a 100-μl aliquot was analyzed by the aforementioned combination of HPLC and UV spectrophotometry. The solution contained a compound which appeared to be reasonably pure by HPLC and had a UV spectrum characteristic of the impurity. The dichloromethane solution was evaporated to dryness under a stream of dry nitrogen. Electron-impact mass spectra for this compound and for 2-mercaptobenzothiazole[14] (I), a compound suspected to be the impurity based on the prevailing evidence, were obtained by direct probe on a mass spectrometer[15].

Comparison of the Impurity to Standard I by HPLC and UV Spectrophotometry—A slice of rubber closure (55 mg) was placed in 5 ml of 0.5 *N* NaOH in a tightly stoppered container and heated at 50° for 24 hr. The solution was diluted with a mixture of CH_3CN–H_2O–HOAc (50:47:3), which produced a solution of pH 7, and injected onto the reverse-phase column. The rubber sheath (125 mg) was treated similarly. The extracted compound was compared to standard I by retention time and by the UV spectrum measured using the technique described above.



I

A mobile phase consisting of CH_3CN–H_2O–HOAc (260:740:0.03) provided an amount of acetic acid that was sufficiently high (the mobile phase was pH 4) to suppress ionization of the compound, but sufficiently low to prevent its interference in the UV spectrum of the compound.

A 100-μl portion of the digoxin injectable solution sample was mixed with 120 μl of the mobile phase, and a 200-μl aliquot was chromatographed. The impurity, which was separated from digoxin, was collected from the column and examined in both acid and base by UV spectrophotometry. Standard I was treated similarly, and its spectra were compared to those of the impurity.

Silica Gel HPLC and UV Spectrophotometry—Four different digoxin (0.25 mg/ml) and two sodium phenobarbital (65 and 130 mg/ml) injectable solution samples were extracted with organic solvents by the method described below to provide samples for silica gel chromatography. Because of its high concentration relative to the contaminant in the injectable solutions, it was necessary to eliminate most of the phenobarbital to avoid column overload and to allow resolution of the impurity from phenobarbital. Both phenobarbital and I are weakly acidic compounds, which eliminated simple base extraction as a means of separation. 1-Chlorobutane had the lowest reported distribution coefficient (K = 0.4) for phenobarbital (2), and therefore it was the solvent of choice for elimination of phenobarbital. Water (4 ml; 8 ml for sodium phenobarbital at 130 mg/ml), 1-chlorobutane (5 ml), the injectable solution (1–1.5 ml), and 0.5 *N* HCl (1 ml) were shaken in a 30-ml separatory funnel. The upper (chlorobutane) layer was separated, washed with four 5-ml portions of 0.5 *N* HCl, and evaporated to dryness. The residue was partitioned between 3 ml of 0.1 *N* NaOH and 3 ml of heptane. The aqueous base was acidified with 1 *N* HCl and extracted with 4 ml of CH_2Cl_2. The organic solvent was evaporated; the residue was dissolved in 200 μl of mobile solvent, and the entire amount was chromatographed. The contaminant was separated on a silica gel column[16] with a mobile phase consisting of heptane–isopropyl alcohol containing 1% water–acetic acid (990:10:0.3). The column effluent was passed through a rapid-scanning spectrophotometer, as previously described.

Examination of Various Injectable Drug Solutions—Samples from several manufacturers of injectable solutions of digoxin, sodium phenobarbital, epinephrine, lidocaine hydrochloride, mepivacaine hydrochloride, pilocarpine hydrochloride, and dexamethasone sodium phosphate, each packaged in a single-dose delivery syringe or syringe cartridge where the solution was in contact with a rubber closure, were examined for the presence of I by reverse-phase HPLC with a mobile phase of CH_3CN–H_2O–HOAc (260:740:0.3). Each injectable solution (200 μl), except for sodium phenobarbital, was used undiluted. With phenobarbital injectable solutions, I was not resolved from the drug during HPLC; therefore, it was necessary to perform a preliminary extraction by the procedure described above.

As each injectable solution was chromatographed, the fraction of eluate containing a compound with a retention time equal to that of standard I was collected in a microcell. The UV spectrum was recorded at pH 4 (the mobile phase pH) and again at pH 11 or 12 after the addition of 10–20 μl of 1 *N* NaOH. Spectra were normalized at their absorbance maxima (323 or 322 nm in acid; 311 or 310 nm in base) for direct comparison to the spectra of the standard.

Quantitation—Each of the various injectable drug solutions was quantitatively analyzed for I. The HPLC system was the same as that used in the analysis of digoxin injectable solution, except that the variable-wavelength detector was set to 323 nm and 0.04 AUFS and the mobile phase consisted of 30% acetonitrile and 1% acetic acid in water. Injectable solution samples were used directly without dilution. The standard solution was prepared by dissolving I in 50% ethanol to give a concentration of 4 or 8 μg/ml. The injection volume for samples and standard was 40 μl. Linearity for I was established. Repeatability was examined by making seven successive injections of I standard solution and measuring peak area. The relative standard deviation was 0.25%.

Interference with Digoxin Assay—Three samples of digoxin injectable solution known to contain I were assayed for digoxin by the HPLC method specified in the USP (1) with 31% CH_3CN, a mobile phase in which I coeluted with digoxin, and with 29% CH_3CN, a mobile phase in which I was separated from digoxin. The percent of the label claim was determined in each case.

RESULTS

Origin and Identity of the Contaminant—The chromatogram of the contaminated digoxin injectable solution is shown in Fig. 1; the mobile

[11] Good spectra were obtained at absorbance readings as low as 0.01 AUFS. At higher concentrations, smooth spectra were obtained with continuous flow through the flowcell.

[12] U6K injector; Waters Associates.

[13] Undoubtedly, this was far in excess of the time required for extraction. The extraction process was simply left unattended.

[14] Aldrich Chemical Co., Milwaukee, WI 53233.

[15] LKB 9000 mass spectrometer; LKB Instruments, Inc., Rockville, MD 20852.

[16] LiChrosorb Si60, 10-μm particle size, 250 mm (length) × 4.6 mm (i.d.); EM Laboratories, Inc., Elmsford, NY 10523.

Figure 1—*Chromatogram of a commercial sample of digoxin injectable solution showing the contaminant, 2-mercaptobenzothiazole (A), and digoxin (B). The mobile phase was 29% acetonitrile; the detector was set at 218 nm.*

phase was 29% acetonitrile. When a mobile phase of 26% acetonitrile was used to obtain the chromatograms of cardiac glycosides related to digoxin, the impurity had a retention time within 0.1 min of that of diginatin. However, in 30% acetonitrile, diginatin and the impurity were well separated with retention times of 5.30 and 7.70 min, respectively.

The UV spectrum of chromatographically purified contaminant had a λ_{max} that varied from 320 to 316 nm, corresponding to slight variations in the mobile phase composition. This implied that the impurity was devoid of an unsaturated butyrolactone chromophore and was unrelated

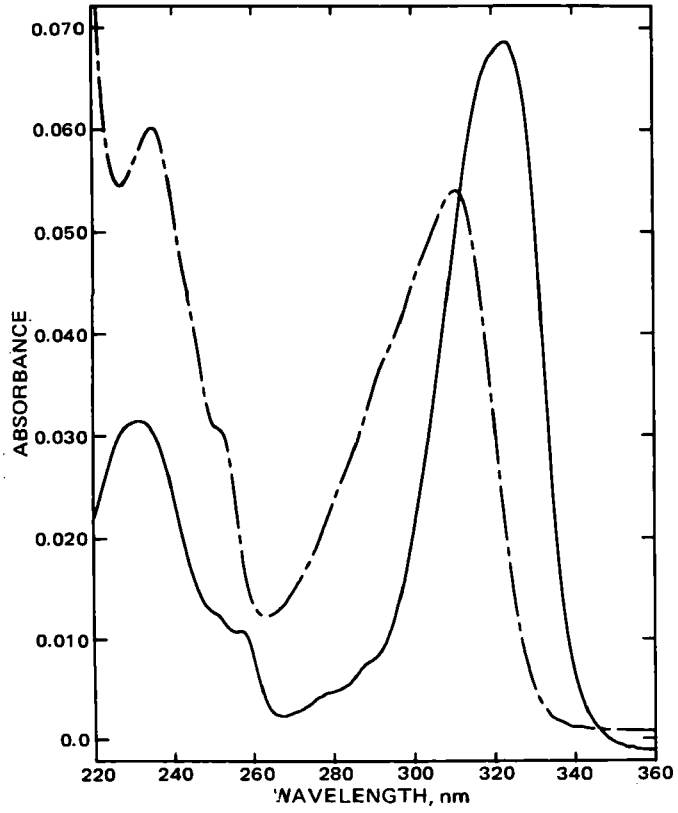


Figure 2—*UV spectra of 2-mercaptobenzothiazole in acetonitrile–water at pH 4 (—) and at pH 12 (— — — — —).*

Table I—Qualitative and Quantitative Analysis of 2-Mercaptobenzothiazole (I) in Various Injectable Drug Solutions [a]

Manufacturer	λ_{max} of Contaminant, nm (pH)[b]	Amount Found, μg/ml[c]
	Standard I	
—	323 or 322 (4); 311 or 310 (12)	—
	Dexamethasone Sodium Phosphate (4 mg/ml)	
A	—	0
B	323 (4); 307 (12)[d]	1.1
B	323 (4); 311 (12)	7.9
	Digoxin (0.25 mg/ml)	
A	323 (4); 311 (11)	8.2
A	323 (4); 311 (12)	4.6, 7.5, 8.6
A	323 (4); 311 (12)	2.9, 6.5, 6.2
A	323 (4); 311 (12)	6.6
	Epinephrine (1:1000)	
A	323 (4); 311 (12)	9.7
A	323 (4); 311 (12)	11.1
A	323 (4); 311 (12)	11.6
	Lidocaine Hydrochloride (2%)	
C[e]	—	0
C	323 (4); 311 (12)[d]	1.0, 0, 0
D	323 (4); 310 (12)	2.8
E[e]	—	0
	Mepivacaine Hydrochloride (2%)	
C	—	0
D	323 (4); 310 (12)	1.5
D	323 (4); 310 (12)	3.5, 3.1, 3.5
	Pilocarpine Hydrochloride (4%)	
F	323 (4); 310 (12)[d]	1.0
F	323 (4); 307 (12)[d]	0.7
	Sodium Phenobarbital (60 mg/ml)	
A	323 (4); 311 (11)	3.2
	Sodium Phenobarbital (130 mg/ml)	
A	323 (4); 311 (12)	3.4
G	323 (4); 311 (12)	5.1

[a] Each injectable solution listed represents a different lot. Some samples were past their expiration date. [b] Unless otherwise noted, normalized spectra were practically superimposable with those of standard I. [c] Multiple values represent individual analyses of injectable solutions of the same lot but from different containers. [d] UV spectrum was distorted especially at shorter wavelengths due to interfering substances. [e] Three lots from this manufacturer were free of I.

to digoxin. When measured as a function of pH, the absorbance maximum shifted from 311 nm in base to 323 nm in acid, with the greatest shift occurring at pH ~6–7, indicating that the compound was ionic or ionizable. The impurity was partitioned from dilute hydrochloric acid into chloroform and from chloroform into aqueous base. This partitioning behavior, in conjunction with the observed pH profile, showed that the impurity was an acidic compound with an estimated pK_a of 6–7.

The rubber sheath and rubber closure extracts contained a compound with a retention time and a UV spectrum that matched those of the impurity in the digoxin injectable solution; the glass barrel extract did not. Thus, the impurity originated from the rubber parts of the syringe, and since the injection medium had limited, if any, contact with the rubber sheath which covered the needle, the contaminant was undoubtedly leached from the rubber closure.

2-Mercaptobenzothiazole (I), a compound commonly used as an accelerator in the vulcanization of rubber, contains a weakly acidic aromatic thiol group[17] and has an absorbance maximum at 320 nm (4), making it a prime candidate for the structure of the contaminant. The electron-impact mass spectrum for standard I and for the compound extracted from the rubber sheath matched very closely, each with a very strong molecular ion at m/z 167 (base peak). The impurity in the digoxin in-

[17] The acidic dissociation constant of I has been determined spectrophotometrically to be 6.93 in 40% aqueous ethanol at 27° (3).

Table II—Assay of Digoxin Injectable Formulation Samples by HPLC With and Without Interference from 2-Mercaptobenzothiazole (I)

	Mobile Phase				
	29% CH_3CN		31% CH_3CN		
Sample	Retention Time, min[a]	Assay[b]	Retention Time, min[c]	Assay[b]	Difference[b]
1	7.55	—			
	8.75	95.3	5.90	106.4	11.1
2	7.30	—			
	8.75	96.1	5.95	116.5	20.4
3	7.15	—			
	8.70	95.7	5.95	106.3	10.6

[a] The first peak was due to the impurity (I); drift in retention time was due to incomplete column equilibration. The compound of longer retention time was digoxin. [b] Percent of label claim. [c] Digoxin and I eluted together as a single peak.

jectable solution and the compounds extracted from the rubber sheath and rubber closure had the same retention time and the same UV spectrum as standard I. The UV spectra of the standard had absorbance maxima at 323 and 311 nm at pH 4 and pH 12, respectively (Fig. 2). These maxima were also oberved for the impurity when treated similarly.

Examination of Other Drug Injectable Solution Samples—Efforts to establish the source and identity of the impurity had been conducted on one digoxin injectable solution sample. Since the contaminant was found to originate from the rubber closure of the injection syringe, one would expect other drug samples packaged in this or a similar type of container to contain the contaminant also. Results of the qualitative and quantitative analysis of all drug injectable formulation samples examined are given in Table I. Fortunately, in most of the drug samples that were examined qualitatively by HPLC and by UV spectrophotometry, the injection ingredients did not interfere with the identification of I.

In some instances, the contaminant peak was not completely resolved from the tail or leading edge of the drug or excipient peaks, and some distortion was evident in the UV spectrum of the collected sample. This distortion occurred only in a few samples containing low levels of I (Table I) and, even in these cases, the distortion occurred generally in the shorter wavelengths of the spectrum; the peak at 323 nm (in acid) was unaffected, and the peak normally at 310 nm (in base) was sometimes shifted slightly toward shorter wavelengths. When there was no chromatographic interference, spectra of the isolated contaminant normalized at their absorbance maxima (323 nm in acid, 311 nm in base) were practically superimposable with those of the standard. In the case of sodium phenobarbital, I was completely submerged under one large phenobarbital absorption peak, necessitating removal of the drug by a preliminary extraction.

During quantitative analysis of the various drug samples when the detector was set to 323 nm, the only peak observed in the chromatogram, other than peaks occasionally seen at or near the solvent front, was the peak corresponding to I. 2-Mercaptobenzothiazole (I) was found in over half of the injectable solutions examined.

Silica Gel HPLC—Silica gel HPLC was performed on four digoxin and two sodium phenobarbital injectable solutions to complement the evidence provided by reverse-phase HPLC. Each sample contained a compound with a retention time between 6.10 and 6.22 min, which compared well with retention times of 6.09 and 6.19 min for duplicate injections of standard I. The spectrum of each sample and standard had an absorbance maximum at 327 nm, although the spectra of the impurity from phenobarbital samples were distorted by the presence of interfering compounds that were poorly resolved by HPLC.

Interference with Digoxin Assay—In reverse-phase HPLC, I was influenced less significantly by changes in the mobile phase than digoxin. Thus, in 29% CH_3CN I eluted before digoxin, in 31% CH_3CN the compounds coeluted, and in 34% CH_3CN digoxin eluted before I. Coelution of these two compounds during digoxin assay would give erroneously high assay values. Table II shows that the true assay results were raised by 10.6, 11.1, and 20.4% for three digoxin samples due to interference from I.

DISCUSSION

After the contaminant in the digoxin injectable formulation was identified, it was thought that this compound, being acidic, would be found at higher concentrations in injectable solutions of high pH, such as sodium phenobarbital; this proved not to be the case. The digoxin injectable solution medium consisted of 40% propylene glycol, 10% alcohol, and 50% water at neutral pH; sodium phenobarbital and dexamethasone sodium phosphate injectable solutions were weakly basic aqueous solutions; and epinephrine, lidocaine hydrochloride, and mepivacaine hydrochloride were weakly acidic aqueous solutions. A comparison of the amounts of I found in the injectable solutions (Table I) showed that the concentration of the impurity had no apparent dependence on the composition or pH of the solution medium and undoubtedly was more dependent on the level of the compound in the rubber closure. The concentration of I varied from lot to lot of a particular drug solution, and even from one container to the next within the same lot.

There are literature reports describing the extraction of I and related compounds from rubber materials. Various types of rubber were extracted with solvents simulating those found in food products, and all but chloroprene were found to contain I, its zinc salt, or 2,2-dithiobis-(benzothiazole), all apparently assayed as I by GC (5). The compound has also been extracted into water from rubber articles that come in contact with foods (6, 7). Two compounds related to I, 2-(methylmercapto)benzothiazole (8) and 2-(2-hydroxyethylmercapto)benzothiazole (9), have been extracted into aqueous media from the rubber closures of disposable syringes. The latter compound reportedly arose from a reaction between I and ethylene oxide, a compound used during the sterilization of the disposable syringes.

The official method of analysis for digoxin injectable solution (1) specifies reverse-phase HPLC with acetonitrile–water as the mobile phase. Unfortunately, if I were present in the digoxin solution, it might elute from the column together with digoxin and raise the apparent analytical result.

A literature survey was conducted for known toxicological properties of I. The compound was shown to be an allergen in rubber-induced skin sensitivity (10, 11). Mutagenic activity of I was observed in fruit flies (12), and slight mutagenic activity was produced in cultured cells from Chinese hamsters by a rubber extract containing I and other vulcanizing accelerators (7). One study revealed that the compound may produce neoplasms in mice (13), although the results of that study were inconclusive, and the amounts of the compound used in that study were much greater than the amounts found in the injectable solutions.

In conclusion, it appears that I is a common contaminant for a variety of injectable drug solutions packaged in single-dose injection syringes or syringe cartridges with rubber closures. It may pose problems as an analytical or toxicological contaminant.

REFERENCES

(1) "The United States Pharmacopeia," 20th rev., second supplement, United States Pharmacopeial Convention, Rockville, Md., 1980, pp. 58–59.

(2) M. K. C. Chao, K. S. Albert, and S. A. Fusari, in "Analytical Profiles of Drug Substances," vol. 7, K. Florey, Ed., Academic, New York, N.Y., 1978, pp. 374–375.

(3) J. P. Danehy and K. N. Parameswaran, *J. Chem. Eng. Data*, **13**, 386 (1968).

(4) "Organic Electronic Spectral Data," vol. IV, J. P. Phillips and F. C. Nachod, Eds., Wiley, New York, N.Y., 1963, p. 102.

(5) G. Salvatore and A. Sampaolo, *Rass. Chim.*, **25**, 54 (1973); through *Chem. Abstr.*, **79**, 77074r (1973).

(6) B. Zyszczynska-Florian, *Rocz. Panstw. Zakl. Hig.*, **25**, 63 (1974); through *Chem. Abstr.*, **81**, 38627v (1974).

(7) T. Baba, *Osaka Shiritsu Daigaku Igaku Zasshi*, **29**, 807 (1980); through *Chem. Abstr.*, **95**, 148799z (1981).

(8) M. A. Inchiosa, Jr., *J. Pharm. Sci.*, **54**, 1379 (1965).

(9) M. C. Petersen, J. Vine, J. J. Ashley, and R. L. Nation, *J. Pharm. Sci.*, **70**, 1139 (1981).

(10) F. Saito and Y. Yamatuta, *Nippon Hifuka Gakkai Zasshi*, **82**, 763 (1972); through *Chem. Abstr.*, **78**, 150970r (1973).

(11) A. A. Anton'ev and I. V. Gerasimenko, *Vestn. Dermatol. Venerol.*, 56 (1976); through *Chem. Abstr.*, **85**, 129775r (1976).

(12) Y. A. Revazova, *Toksikol. Nov. Khim. Veshchestv, Vnedryaemykh Rezin. Shinnuyu Prom.*, 196 (1968); through *Chem. Abstr.*, **71**, 47071e (1969).

(13) National Technical Information Service, PB 223-159; through "Registry of Toxic Effects of Chemical Substances," 1978 ed., R. J. Lewis, Sr., Ed., National Institute for Occupational Safety and Health, Cincinnati, Ohio, 1979, p. 228.