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High-performance liquid chromatographic assay of thiomersal (thimerosal) as the ethylmercury dithiocarbamate complex

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ABSTRACT

A high-performance liquid chromatographic (HPLC) assay has been developed for the ethylmercury ligand of thiomersal (thimerosal) as either the morpholine or piperidinedithiocarbamate complexes. The assay has been compared with a previously reported HPLC assay of thiomersal for organomercurical degraded both thermally and photochemically, and significant differences are noted.

INTRODUCTION

Thiomersal (thimerosal, TM), the sodium salt of the complex formed between thiosalicylic acid (TSA) and ethylmercury (EtHg) (compound I, Fig. 1), is used as a

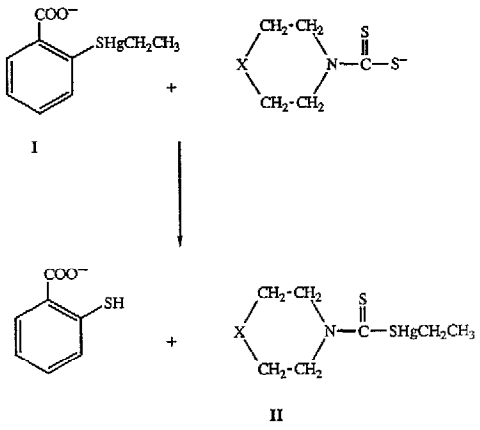

Fig. 1. Reaction of MDTC (X = O) and PIDTC (X = CH_2) with thiomersal (I) to form thiosalicylic acid and the MDTC or PIDTC complex of ethylmercury (II).

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topical antiseptic and antimicrobial preservative [1]. It is unstable to light [2–4] and can be adsorbed by plastics [4–6] both of which influence the potential shelf-life of pharmaceutical products. In studies of these problems and for routine analytical purposes a number of analytical methods have been reported using colorimetry [3,5,7], atomic absorption spectroscopy [8,9], polarography [10] and high-performance liquid chromatography (HPLC) [2–4, 11–14]. In studies where these analytical methods have been compared using identical degraded samples of TM, considerable differences have been noted [2–4].

In keeping with other mercurial antiseptics and antimicrobial preservatives the antimicrobial activity of TM probably resides in the EtHg ligand [15–17]. Should the two components of the complex (TSA and EtHg) decompose at different rates assessment of total EtHg (intact TM and free EtHg) may afford a better chemical assessment of active antimicrobial activity. All the reported HPLC methods are designed to detect and quantitate intact TM cognizance having not been given to the consequence of losses of TSA resulting in a reduced level of measured TM due to changes in the stoichiometric ratio of TSA and EtHg in the mixture. It is known that the major decomposition products of TM are 2,2′-dithiosalicyclic acid formed by oxidation of TSA, EtHg and, in the presence of light, elemental mercury [4]. Further, it has been shown that degraded samples of TM possess enhanced levels of antimicrobial activity, and it has been suggested that this may be due to EtHg formed during degradation [17].

The development in these laboratories of a range of dithiocarbamate (DTC)-complexing agents and their application to the quantitation of phenylmercuric nitrate [18–20] makes possible the development of an EtHg ligand-specific assay for TM. This paper reports the development of two such assays using morpholinedithiocarbamate (MDTC) and piperidinedithiocarbamate (PIDTC) and compares the results of this type of assay to the conventional HPLC method [13] using thermally and photochemically degraded samples of TM.

EXPERIMENTAL

Materials

TM and TSA (Sigma, St. Louis, MO, U.S.A.) and EtHgCl (TCI, Tokyo, Japan) were used as supplied. All other chemicals were analytical or HPLC grade. The morpholinium salt of MDTC and piperidinium salt of PIDTC were prepared as reported previously [18,19].

Preparation of derivatives

The MDTC and PIDTC reagents were prepared by dissolving 10, 20, 60 and 100 mg of the corresponding salts in 100 ml of acetonitrile–water (75:25) and 100% acetonitrile, respectively. Of this reagent, 1 ml was added to 1 ml of sample in a glass vial followed by mixing. For routine analytical purposes a concentration of 60 mg of reagent salt per 100 ml of solvent is appropriate.

Chromatographic equipment and conditions

The liquid chromatograph consisted of a Model 501 pump (Waters Assoc., Milford, MA, U.S.A.), Rheodyne Model 7125 loop injector (Cotati, CA, U.S.A.),

Model 484 variable-wavelength absorbance detector (Waters Assoc.) and Model 3396A integrating recorder (Hewlett-Packard, Palo Alto, CA, U.S.A.) together with a column of octadecyl silica (Waters Assoc.), 30 cm × 3.9 mm I.D., 10 μm particle size. The solvent consisted of $1 \cdot 10^{-4}$ *M* disodium ethylenediaminetetraacetate in 70 or 85% methanol (for the MDTC and PIDTC reagents, respectively) at a flow-rate of 1.5 ml min^{-1} and monitoring at 258 nm. The injection volume used was 20 μl.

Degradation of Thiomersal

Samples of TM at a concentration of 0.01% (w/v) were submitted to degradation in Pyrex flasks in direct sunlight in the presence or absence of 0.5% (w/v) sodium chloride or under reflux in 0.5% sodium chloride either protected from light or in subdued room lighting. Samples were withdrawn at regular intervals for analysis.

RESULTS AND DISCUSSION

The addition of excess DTC-complexing reagents to a solution of TM leads to rapid ligand exchange to form either the EtHg–MDTC or the EtHg–PIDTC com-

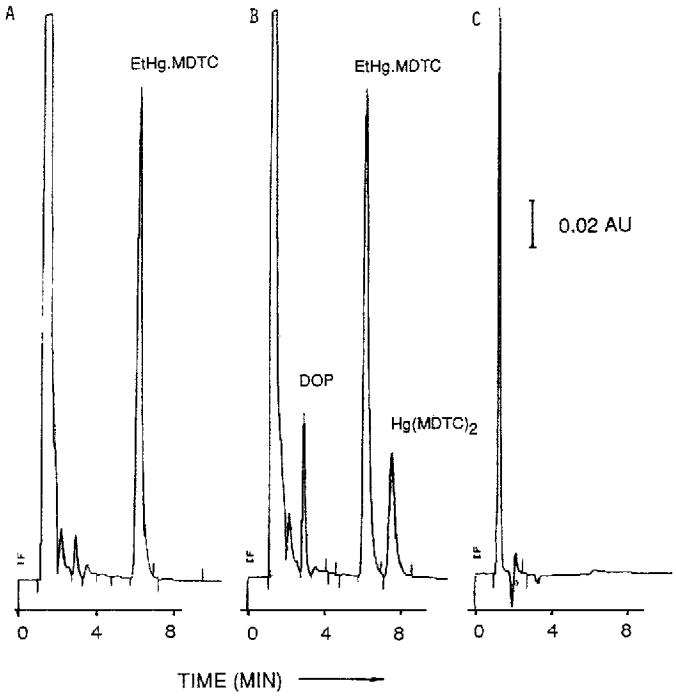

Fig. 2. Chromatograms of thiomersal assay using morpholinedithiocarbamate. (A) $6 \cdot 10^{-3}$% (w/v) thiomersal (1 ml) plus 0.06% (w/v) MDTC reagent (1 ml); (B) $6 \cdot 10^{-3}$% (w/v) thiomersal containing $1 \cdot 10^{-3}$% (w/v) mercuric acetate (1 ml) plus 0.06% (w/v) MDTC reagent (1 ml); (C) $6 \cdot 10^{-3}$% (w/v) thiomersal diluted 1:2 with acetonitrile. Peaks: 6.16 min, EtHg–MDTC; 7.48 min, $Hg(MDTC)_2$; 2.87 min, disulphide oxidation product (DOP) of derivatising agent. Excess MDTC thiosalicylic acid and dithiosalicylic acid elute at the void volume (1.5 min).

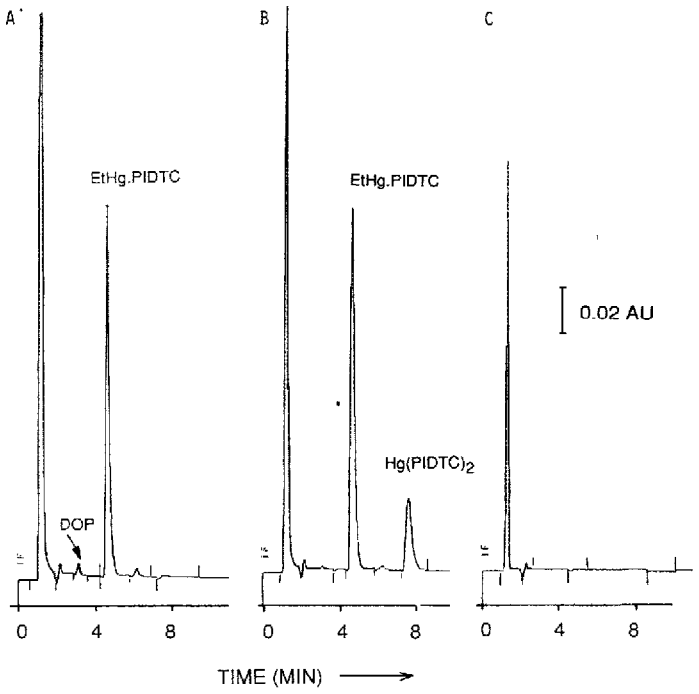

Fig. 3. Chromatograms of thiomersal assay using piperidinedithiocarbamate. (A) $6 \cdot 10^{-3}$% (w/v) thiomersal (1 ml) plus 0.06% (w/v) PIDTC reagent (1 ml); (B) $6 \cdot 10^{-3}$% (w/v) thiomersal containing $1 \cdot 10^{-3}$% (w/v) mercuric acetate (1 ml) plus 0.06% (w/v) PIDTC reagent (1 ml); (C) $6 \cdot 10^{-3}$% (w/v) thiomersal diluted 1:2 with acetonitrile. Peaks: 4.67 min, EtHg–PIDTC; 7.49 min, $Hg(PIDTC)_2$; 3.15 min, disulphide oxidation product (DOP) of derivatising agent; 6.08 min, unidentified contaminant of PIDTC agent which does not interfere with the assay. Excess PIDTC, thiosalicylic acid and dithiosalicylic acid elute at the void volume (1.5 min).

plexes (compound II, Fig. 1) which elute as clean peaks without interference (Figs. 2 and 3). The identity of the peaks ascribed to EtHg–MDTC, EtHg–PIDTC and TSA have been confirmed with an authentic sample of TSA and with the complexes formed between EtHg–Cl and MDTC and PIDTC reagents. Previous studies of the stability of TM have demonstrated the presence of elemental mercury as a major degradation product [4] and, under the chromatographic conditions employed in these assays, the $Hg(MDTC)_2$ and $Hg(PIDTC)_2$ complexes do not coelute with the peaks arising from the EtHg–DTC complexes (Figs. 2B and 3B). Also shown are the chromatographic traces obtained following dilution of TM with acetonitrile without the addition of derivatising agents (Figs. 2C and 3C). Disodium ethylenediaminetetraacetate has been added in low concentration to the HPLC mobile phases to minimise the possiblity of interference from other metal ions in the system [21].

Both reagents afford a linear relationship between area response and concentration which pass through the origin for the concentration range 0–$1.2 \cdot 10^{-2}$% (w/v) of TM and allow quantitation of TM with reasonable precision (Table I). To show that the reaction proceeds rapidly to completion, studies have been undertaken to confirm that the peak-area responses of the EtHg–DTC complexes are indepen-

TABLE I

DATA FROM ASSAYS OF THIOMERSAL

Area response = slope × concentration (%, w/v) + intercept.

Compound	Slope	Concentration range (%, w/v)	Intercept	r	n	C.V. at $6 \cdot 10^{-3}$% (w/v) ($n = 6$) (%)
MDTC	$1.298 \cdot 10^9$	0–$1.2 \cdot 10^{-2}$	$7.73 \cdot 10^4$	0.9998	7	0.82
PIDTC	$1.316 \cdot 10^9$	0–$1.2 \cdot 10^{-2}$	$1.038 \cdot 10^5$	0.9998	7	0.65

TABLE II

AREA RESPONSE AS A FUNCTION OF THE TIME FOLLOWING MIXING

Data obtained using PIDTC reagent; thiomersal concentration was 0.01% (w/v).

Time following mixing (min)	Area response relative to that at 0.5 min
0.5	1.000
2.0	0.999
5.0	0.992
10.0	1.003

TABLE III

AREA RESPONSE AS A FUNCTION OF REAGENT CONCENTRATION

Data obtained using MDTC reagent; thiomersal concentration was 0.01% (w/v).

Concentration (%, w/v)	Response relative to that at 0.060% ((w/v)
0.010	0.989
0.020	1.005
0.060	1.000
0.100	0.996

dent of both complexing reagent concentration [0.01–0.1% (w/v) MDTC] (Table II) and time following mixing (0.5–10.0 min) (Table III). The complexation reaction occurs rapidly, the complex, once formed, is stable and the formation is independent of the concentration of complexing reagent [0.01–0.1% (w/v) corresponds to a 1.6–16 M excess of complexing agent]. For all subsequent studies a concentration of 0.06% (w/v) of complexing agent was used which corresponds to a ten-fold excess when TM is present at a concentration of 0.01% (w/v) in the sample.

To assess the assay, it has been compared with the previously reported HPLC method of Lam *et al.* [13] for samples degraded both thermally and photochemically

TABLE IV

COMPARISON OF LITERATURE HPLC ASSAY FOR THIOMERSAL WITH THIS ASSAY PROCEDURE

Time (h)	Percent remaining	
	Assay using PIDTC	Literature assay [13]
Under reflux in 0.5% sodium chloride in darkness		
0	100.0	100.0
5	99.6	98.3
20	99.2	97.8
24	100.2	96.8
Under reflux in 0.5% sodium chloride in subdued lighting		
0	100.0	100.0
5.5	98.3	95.1
20	95.7	85.1
25	92.9	78.5
Direct sunlight in plain solution		
0	100.0	100.0
0.25	96.3	96.7
0.5	89.6	88.5
1.0	72.6	71.0
2.0	37.6	30.3
Direct sunlight in 0.5% sodium chloride		
0	100.0	100.0
0.25	97.5	95.9
0.5	91.6	85.0
1.0	79.2	57.3
2.0	54.0	<1.0

(Table IV). A fully degraded sample of TM (direct sunlight for 12 h) when submitted to both methods of analysis afforded a flat baseline at the retention time of the EtHg–DTC complexes thus demonstrating that the degradation products do not interfere with either analytical method.

Refluxing in darkness in 0.5% sodium chloride indicated that the EtHg is stable thermally but the method of Lam *et al.* [13] demonstrated losses of TM. This is probably due to the slow atmospheric oxidation of the TSA to 2,2'-dithiosalicylic acid [4] altering the stoichiometry of the mixture of EtHg and TSA. When refluxed in subdued lighting losses of EtHg also occurred but at a considerably lower rate than indicated for TM by the literature method.

When TM solution was placed in direct sunlight destruction was rapid. Previous studies have suggested that halide ions promote the photochemical destruction of TM [22–24] and this is reflected in the results obtained using the HPLC method of Lam *et al.* [13]. However, when EtHg is measured using the DTC reagent it is demonstrated that the presence of the chloride enhances the stability of the EtHg ligand probably due to its tendency to complex with the organomercury ligand to form

EtHg–Cl [25,26]. The displaced TSA may then undergo more rapid decomposition by oxidation.

From these observations it is obvious that TM may be measured as EtHg and that the results obtained differ markedly from a more conventional method in which TM is quantitated directly. The availability of at least two alternative DTC-complexing agents makes possible the selection of reagents which may avoid interference from product components in more complex pharmaceutical formulations, and these assays may more correctly reflect the true antimicrobial activity of the TM in such formulations.

REFERENCES

1 R. G. Kimpton, *Aust. J. Hosp. Pharm.*, 17 (1987) 191.
2 B. J. Meakin and Z. M. Khammas, *J. Pharm. Pharmacol.*, 30 (1978) 52P.
3 S. N. Ibrahim, N. Stroud and B. J. Meakin, *J. Pharm. Pharmacol.*, 33 (1981) 7P.
4 M. J. Reader and C. B. Lines, *J. Pharm. Sci.*, 72 (1983) 1406.
5 N. E. Richardson, D. J. G. Davies, B. J. Meakin and D. A. Norton, *J. Pharm. Pharmacol.*, 29 (1977) 717.
6 C. M. McTaggart, J. Ganley, T. Eaves, S. E. Walker and M. J. Fell, *J. Pharm. Pharmacol.*, 31 (1979) 60P.
7 A. R. Neurath, *Cesk. Farm.*, 10 (1961) 75; *C.A.*, 61 (1964) 11854 g.
8 B. J. Meakin and Z. M. Khammas, *J. Pharm. Pharmacol.*, 31 (1979) 653.
9 W. Holak, *J. Assoc. Off. Anal. Chem.*, 66 (1983) 1203.
10 S. Pinzauti and M. Casini, *Farmaco, Ed. Prat.*, 35 (1980) 92; *Anal. Abstr.*, 39 (1980) 3E 66.
11 C. C. Fu and M. J. Sibley, *J. Pharm. Sci.*, 66 (1977) 738.
12 R. C. Meyer and L. B. Cohn, *J. Pharm. Sci.*, 67 (1978) 1636.
13 S. W. Lam, R. C. Meyer and L. T. Takahashi, *J. Parenter. Sci. Technol.*, 35 (1981) 262.
14 G. C. Visor, R. A. Kenley, J. S. Fleitman, D. A. Neu and I. W. Partridge, *Pharm. Res.*, 2 (1985) 73; *Anal. Abstr.*, 48 (1986) 3E 53.
15 A. R. Martin, in R. F. Doerge (Editor), *Wilson and Gisvold's Textbook of Organic, Medicinal and Pharmaceutical Chemistry*, Lippincott, Philadelphia, PA, 8th ed., p. 145.
16 V. M. Trikojus, *Nature (London)*, 158 (1946) 472.
17 D. J. G. Davies, Y. Anthony and B. J. Meakin, *Expo. Congr. Int. Technol. Pharm. 3rd*, 4 (1983) 238.
18 J. E. Parkin, *J. Chromatogr.*, 407 (1987) 389.
19 J. E. Parkin, *J. Chromatogr.*, 472 (1989) 401.
20 J. E. Parkin, *J. Chromatogr.*, 511 (1990) 233.
21 S. R. Hutchins, P. R. Haddad and S. Dilli, *J. Chromatogr.*, 252 (1982) 185.
22 K. Horworka, B. Horworka and R. Meyer, *Pharmazie*, 28 (1973) 136.
23 E. Lüdtke, H. Darsow and R. Pohloudek-Fabini, *Pharmazie*, 32 (1977) 99.
24 E. Lüdtke and R. Pohloudek-Fabini, *Pharmazie*, 32 (1977) 625.
25 R. B. Simpson, *J. Am. Chem. Soc.*, 83 (1961) 4711.
26 D. L. Rabenstein, *Acc. Chem. Res.*, 11 (1978) 100.