Journal of Chromatography, 587 (1991) 329–332
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 717

Short Communication

Assay for thiomersal (thimerosal) with adaptation to the quantitation of total ethylmercury available in degraded samples

J. E. Parkin

School of Pharmacy, Curtin University of Technology, Kent Street, Bentley, Western Australia 6102 (Australia)

(First received May 16th, 1991; revised manuscript received August 29th, 1991)

ABSTRACT

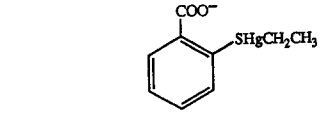
A simple adaptation of a previously reported chromatographic procedure for thiomersal (thimerosal) is reported. This involves the sequential analysis of a degraded sample of thiomersal with and without the addition of excess thiosalicylic acid. This enables the total amount of available ethylmercuric ion and thiomersal to be quantitated and by difference the amount of free ethylmercuric ion. The antimicrobial significance of the results is briefly discussed.

INTRODUCTION

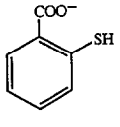
Thiomersal (thimerosal) (TM) (I, Fig. 1) is an antimicrobial preservative used in pharmaceutical systems [1]. It consists of the sodium salt of a complex formed between thiosalicylic acid (TSA) (II) and the ethylmercuric (EtHg) ion (III). It is rapidly degraded by light [2–9] and among the identified degradation products are TSA [2,10–12] 2,2′-dithiosalicylic acid [10–13] and EtHg complexed with various species such as chloride (if present), hydroxide and with excess TM via the carboxylate group [2] and metallic mercury [11].

Davies *et al.* [7] have demonstrated that degraded samples of TM possess enhanced levels of antimicrobial activity, and, on the basis of demonstrating that disodium edetate decreased the activity, have suggested that the EtHg ion is the more active antimicrobial species in degraded samples. This suggestion is in keeping with the proposed mechanism-of-action of mercurial antiseptics [14].

A number of assays employing high-performance liquid chromatography (HPLC) have been applied to the quantitation of TM [3,6,9,11,12,15–19]. All,



I



II

III

Fig. 1. Structures. I = Thiomersal (TM); II = thiosalicylic acid (TSA); III = ethylmercuric ion (EtHg).

0021-9673/91/$03.50

except one recently reported from these laboratories [9], measure intact TM without consideration of the relative levels of EtHg ion and TSA ligand in the system, the measured concentrations, in degraded samples, being limited by the stoichiometric ratio of the two species in solution.

The assay reported from these laboratories [9] quantitates TM (in undegraded samples) or total EtHg ion (in degraded samples) by reaction of the EtHg ion species in the system with dithiocarbamate complexing agents. By applying the analytical method in conjunction with the conventional method of Lam *et al.* [17], which measures intact TM, it is possible to quantitate, in degraded samples, the relative amounts of TM and free EtHg ion by difference. A disadvantage of this procedure is that it requires the application of two HPLC assays concurrently each requiring different chromatographic conditions.

This paper outlines a simple adaptation of the method of Lam *et al.* [17] which enables intact TM and TM plus EtHg ion to be measured using a single HPLC system by sequential assays.

EXPERIMENTAL

Materials

TM and TSA (Sigma, St. Louis, MO, USA) and EtHg chloride (TCI, Tokyo, Japan) were used as supplied. All other chemicals were analytical or HPLC grade.

Chromatographic equipment and conditions

The liquid chromatograph consisted of a pump (501, Waters Assoc., Milford, MA, USA), 20-μl loop injector (Rheodyne 7125, Cotati, CA, USA), variable-wavelength absorbance detector (484, Waters Assoc.) and integrating recorder (3396A, Hewlett-Packard, Palo Alto, CA, USA) together with a column of octadecyl silica (Waters Assoc.) 30 cm × 3.9 mm I.D., 10 μm particle size. The mobile phase consists of 2.74 g of monosodium phosphate and 2.49 g of disodium phosphate in acetonitrile–water (15:85, v/v) at a flow-rate of 1.5 ml min^{-1}. The monitoring wavelength was 254 nm.

Sample preparation

To 1 ml of sample to be analysed was added either acetonitrile–water (30:70, v/v) (1 ml) or an 0.02% (w/v) solution of TSA in acetonitrile–water (30:70 v/v) (1 ml). The solutions were mixed and analysed immediately.

Standard curve for TM

Freshly prepared solutions of TM (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, $3.5 \cdot 10^{-4}$ *M*) were submitted to analysis. The coefficient of variation (C.V.) at $1.5 \cdot 10^{-4}$ *M* was determined using 6 replicate determinations.

Standard curve for EtHg chloride

Freshly prepared solutions of EtHg chloride (0.4, 0.8, 1.2, 1.6 and $2.0 \cdot 10^{-4}$ *M*) were submitted to analysis. The C.V. at $1.2 \cdot 10^{-4}$ *M* was determined using 6 replicate determinations.

Effect of sodium chloride on assay for TM

Freshly prepared solutions of TM ($1.5 \cdot 10^{-4}$ *M*) with and without sodium chloride (0.9%, w/v) were submitted to analysis involving addition to excess TSA.

Comparison of results for assay with that of dithiocarbamate method

A freshly prepared solution of TM ($1.5 \cdot 10^{-4}$ *M*) plus EtHg chloride ($0.1 \cdot 10^{-4}$ *M*) was submitted to analysis by both the assay involving addition of excess TSA and the previously reported method involving piperidinedithiocarbamate [9] using TM as the standard.

Photodegradation of TM

A sample of TM (0.01%, w/v; $2.47 \cdot 10^{-4}$ *M*) in sodium chloride (0.9%, w/v) in a borosilicate glass flask was stored in direct sunlight and samples were withdrawn at 15, 30, 45, 60, 75, 90, 105 and 120 min and immediately submitted to HPLC analysis by both methods. A sample photodegraded for 60 min was submitted to analysis for total EtHg ion by both the method reported here and that using piperidinedithiocarbamate [9].

RESULTS AND DISCUSSION

The chromatographic conditions employed were identical to those of Lam *et al.* [17]. The method differs by preparing samples either by dilution (1:1) with acetonitrile–water (30:70 v/v) which enables TM to be quantitated or by dilution (1:1) with ace-

TABLE I

DATA FOR ASSAYS OF THIOMERSAL AND ETHYLMERCURIC CHLORIDE

Area response = slope × concentration (M) + intercept.

	Slope × 10^{10}	Intercept × 10^{6}	r	n	Concentration range (M)
TM without excess TSA	3.44	−0.184	0.9998	7	0–3.5 · 10^{-4}
TM with excess TSA	3.48	−0.233	0.9997	7	0–3.5 · 10^{-4}
EtHg chloride with excess TSA	3.39	−0.123	0.9998	5	0–2.0 · 10^{-4}

tonitrile–water (30:70, v/v) containing TSA (0.02%, w/v) which quantitates TM plus EtHg ion (Fig. 2). At this concentration of TSA, there is a 5.25-fold excess of TSA to EtHg (when TM is present at a nominal concentration of 0.01% (w/v) (2.47 · 10^{-4} M) and total available EtHg ion is the limiting factor of the stoichiometric ratio of species present. The high affinity of the organomercuric ion for thiol groups assures quantitative conversion of the EtHg ion from its various complexed forms in the system to the TM complex [20,21].

When both diluent systems were applied to freshly prepared standards of TM identical calibration lines were obtained for the assays with and without TSA and for EtHg chloride with TSA (Table I). The C.V. values at 1.5 · 10^{-4} M for TM with and without TSA were ± 1.02% and 0.87%, respectively, and for EtHg chloride the C.V. was ± 1.72% at a concentration of 1.2 · 10^{-4} (n=6 in all cases).

The assay suffers no interference from sodium chloride, a common component of pharmaceutical products which is known to complex with the EtHg ion [mean and C.V. for TM (1.5 · 10^{-4} M) with and without sodium chloride (0.9%, w/v) (n=4): $\bar{x}$ = 1.48 · 10^{-4} M ± 0.48% and $\bar{x}$ = 1.51 · 10^{-4} M ± 0.62%]. The assay involving the addition of excess TSA affords identical results for samples containing TM (1.5 · 10^{-4} M) plus EtHg chloride (0.1 · 10^{-4} M) when compared with the previously reported method involving chromatography of the piperidinedithiocarbamate complex of total EtHg ion [9] [mean and C. V. (n = 4) for TSA assay; 1.58 · 10^{-4} M; ± 1.10% and for the piperidinedithiocarbamate assay: 1.59 · 10^{-4} M; ± 0.92%], the samples being compared against TM as a standard.

When photodegraded samples of TM were submitted to analysis both with and without excess TSA results were obtained demonstrating the presence of substantial amounts of free EtHg ion (Fig. 3). A photodegraded sample (sunlight for 1 h) of

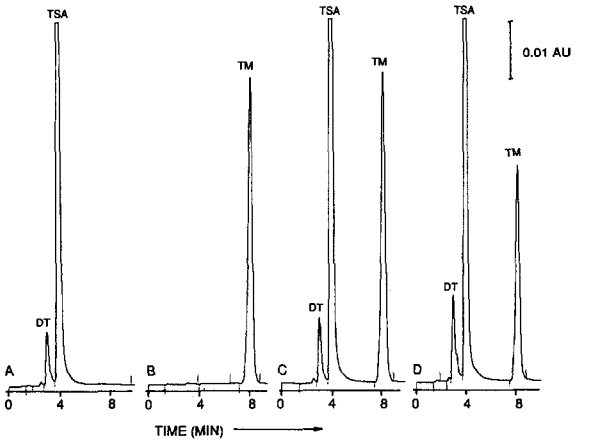


Fig. 2. Representative chromatograms derived by application of the assay. (A) TSA blank; (B) TM (3 · 10^{-4} M) without excess TSA; (C) TM (3 · 10^{-4} M) with excess TSA; (D) EtHg chloride (2 · 10^{-4} M) with excess TSA. DT = 2,2′-Dithiosalicylic acid contaminant in the thiosalicylic acid; TSA = thiosalicylic acid; TM = thiomersal.

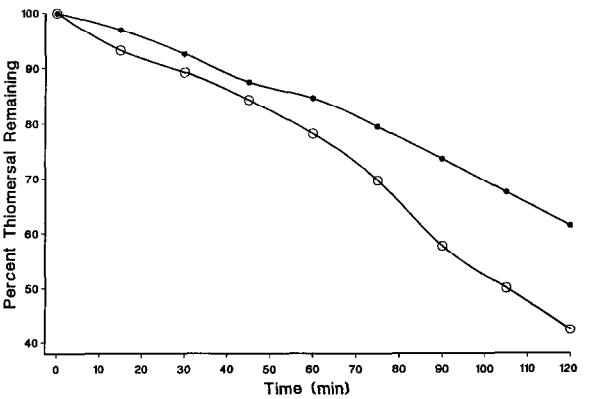


Fig. 3. Results of a typical experiment involving photodegradation of thiomersal in direct sunlight. ● = Concentration of TM with excess TSA; ○ = concentration of TM without excess TSA. The difference represents the amount of free EtHg ion.

TM (0.01%, w/v) when analysed by both the previously reported dithiocarbamate assay and the assay involving the addition of excess TSA gave identical results ($8.52 \cdot 10^{-3}$ and $8.44 \cdot 10^{-3}$%, w/v, remaining respectively calculated as TM). Under the conditions used for this degradation no free TSA was formed. This would be expected if excess EtHg ion were present. With consideration to the mode-of-action of antibacterial organomercurials [7,14] the formation of significant levels of excess EtHg ion would account for the enhanced antimicrobial activity of degraded samples of TM and the assay involving addition of TSA may more correctly reflect analytically the level of antimicrobial activity.

In conclusion, it should be noted that this simple adaptation applied to the method of Lam *et al.* [17] should be readily adaptable to a number of the other reported HPLC methods.

REFERENCES

1 *The Pharmaceutical Codex*, The Pharmaceutical Press, London, 11th ed., 1979, p. 940.
2 F. Tanaka and M. Mitsuno, *Ann. Rept. Takeda Res. Lab.*, 10 (1951) 65; C.A., 47 (1953) 4860 c.
3 B. J. Meakin and Z. M. Khammas, *J. Pharm. Pharmacol.*, 30 (1978) 52P.
4 C. M. McTaggart, J. Ganley, T. Eaves, S. E. Walker and M. J. Fell, *J. Pharm. Pharmacol.*, 31 (1979) 60P.
5 S. Pinzanti and M. Casini, *Farmaco, Ed. Prat.*, 35 (1980) 92; *Anal. Abstr.*, 39 (1980) 3E66.
6 S. N. Ibrahim, N. Stroud and B. J. Meakin, *J. Pharm. Pharmacol.*, 33 (1981) 7P.
7 D. J. G. Davies, Y. Anthony and B. J. Meakin, *Expo. – Congr. Int. Technol. Pharm. 3rd.*, 4 (1983) 238.
8 I. Ahmed. *Pharm. Res.*, 6 (1989) S19.
9 J. E. Parkin, *J. Chromatogr.*, 542 (1991) 137.
10 V. M. Trikojus, *Nature (London)*, 158 (1946) 472.
11 M. J. Reader and J. B. Lines, *J. Pharm. Sci.*, 72 (1983) 1406.
12 G. Anderman, J. Spittler and P. Jonvell, *Labo–Pharma —Probl. Techn.*, 32 (1984) 838, *Int. Pharm. Abstr.*, 25 (1988) 2501773.
13 E. Lüdtke and R. Pohloudek-Fabini, *Pharmazie*, 32 (1977) 625.
14 A. E. Elkhouly and R. T. Yousef, *J. Pharm. Sci.*, 63 (1974) 681.
15 C.-C. Fu and M. J. Sibley, *J. Pharm. Sci.*, 66 (1977) 738.
16 R. C. Meyer and L. B. Cohn, *J. Pharm. Sci.*, 67 (1978) 1636.
17 S. W. Lam, R. C. Meyer and L. T. Takahashi, *J. Parenter. Sci. Technol.*, 35 (1981) 262.
18 M. J. Reader, *J. Pharm. Sci.*, 73 (1984) 840.
19 G. C. Visor, R. A. Kenley, J. S. Fleitman, D. A. Neu and I. W. Partridge, *Pharm. Res.*, 2 (1985) 73.
20 R. B. Simpson, *J. Am. Chem. Soc.*, 83 (1961) 4711.
21 D. L. Rabenstein, *Acc. Chem. Res.*, 11 (1978) 100.