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The Decomposition of Phenylmercuric Nitrate in Sulphacetamide Drops During Heat Sterilization

J. E. PARKIN

School of Pharmacy, Curtin University of Technology, PO Box U1987, Perth, Western Australia 6001

Abstract—Analytical methods have been developed which enable phenylmercuric nitrate, mercuric ion and diphenylmercury to be measured in sulphacetamide drops. Application of these methods demonstrate that during heat sterilization of both 10 and 30% sulphacetamide drops containing phenylmercuric nitrate, together with disodium edetate and sodium metabisulphite, there is a 20–30% overall loss of phenylmercuric nitrate. This loss occurs mainly due to the phenylmercuric ion establishing an equilibrium with equimolar amounts of mercuric ion and diphenylmercury.

Sulphacetamide ophthalmic drops of the Australian Pharmaceutical Formulary and Handbook (1988) are similar to those of The Pharmaceutical Codex (1979) in that they are preserved by phenylmercuric nitrate (0·002% w/v) and also contain disodium edetate (EDTA) and sodium metabisulphite. Phenylmercuric nitrate has been shown to be incompatible with sodium metabisulphite under a variety of conditions (Buckles et al 1971; Richards & Reary 1972; Richards et al 1972; Hart 1973; Collins et al 1985; Parkin 1990, 1991), the phenylmercuric ion being degraded to metallic mercury and, in simple solutions of the two components, to benzenesulphonic and benzenesulphinic acids with diphenylmercury being formed as a transient intermediate (Parkin 1990). EDTA has also been shown to be incompatible with phenylmercuric nitrate in acidic solution with the phenylmercuric ion being degraded to benzene and Hg^{2+} (Parkin et al 1992a, b).

In a study of the stability of phenylmercuric nitrate in ophthalmic products of the APF, Parkin & Marshall (1990) demonstrated that 20–30% of the compound was lost when sulphacetamide drops were heated under the recommended conditions of heat sterilization. The nature of this degradation was not elucidated due to analytical difficulties arising from the high concentration of sulphacetamide sodium in these formulations, but subsequent studies showed an initial rapid decline in the amount of available phenylmercuric nitrate when the drops were sterilized by filtration and stored at room temperature (Marshall & Parkin 1991).

This paper reports the development of an analytical method involving the formation of dithiocarbamate complexes of phenylmercury and Hg^{2+}, followed by selective extraction of these and diphenylmercury into the upper phase of a two-phase system formed by adding sodium chloride and acetonitrile to the sample. This was followed by quantitation by HPLC. Application of these methods to sulphacetamide drops demonstrates that the phenylmercuric nitrate rapidly undergoes the establishment of an equilibrium between phenylmercuric ion and Hg^{2+} and diphenylmercury and confirms the level of loss of phenylmercuric nitrate noted in the earlier study.

Materials and Methods

Materials

Phenylmercuric nitrate (BDH, UK), diphenylmercury (Fluka, Switzerland) and $HgCl_2$ (Ajax Chemicals, Australia) were used as obtained. All other chemicals were of analytical reagent grade or of pharmacopoeial quality. The diethylammonium salt of diethylaminedithiocarbamate (DEADTC) and piperidinium salt of piperidinedithiocarbamate (PIDTC) were synthesized as reported previously from the corresponding amine and carbon disulphide (Parkin 1990). The nonyl 3, 5-dinitrobenzoate used as an internal standard was prepared from the alcohol and 3,5-dinitrobenzoyl chloride by the method of Mann & Saunders (1960). All solvents were HPLC grade.

Equipment and chromatographic conditions

The HPLC system consisted of a pump (501, Waters Associates, USA), a 20-μL loop injector (Rheodyne 7125, USA) a variable wavelength detector (484, Waters Associates) and integrating recorder (3396 series II, Hewlett-Packard, USA) with a column of octadecyl silica 30 cm × 3·9 mm i.d., 10 μm particle size (Waters Associates). Phenylmercuric nitrate and Hg^{2+} were monitored at 258 nm and diphenylmercury at 226 nm. Peak identification was performed using a photodiode-array absorbance detector (Model 991, Waters Associates). The solvent used was methanol–water (80:20) containing 10^{-4} M EDTA at a flow-rate of 1·8 mL min^{-1} and the injection volume was 20 μL.

The gas chromatographic system consisted of a gas chromatograph (5890 Series II, Hewlett-Packard) with a mass-selective detector (5971, Hewlett-Packard) and autosampler (7673, Hewlett-Packard). Selective-ion monitoring was performed at m/z 77. The column consisted of cross-linked 5% phenyl methyl silicone 12 m × 0·2 mm of 0·33 μm thickness at an initial temperature of 100°C programmed to rise at 4°C min^{-1} to 240°C. The injection volume was 1 μL.

Method of analysis

To a 20-mL glass tube with a screw-cap and PTFE wad were added 5 mL of the sample to be analysed, 2 mL saturated

solution of sodium chloride, 1 mL dithiocarbamate salt in water (0·2%, w/v) (PIDTC for phenylmercuric ion and Hg^{2+} and DEADTC for diphenylmercury) and 5 mL 2×10^{-3}% w/v nonyl 3,5-dinitrobenzoate in acetonitrile. The tube was shaken and a sample of the upper phase submitted to analysis by HPLC. The 30% sulphacetamide drops were diluted 2 to 5 mL with water before analysis.

Preparation and treatment of ophthalmic drops

Sulphacetamide drops 10 and 30% w/v were made with the phenylmercuric nitrate being added last with analytical accuracy, from a common stock solution of 0·01% w/v. These drops were assayed for phenylmercuric nitrate immediately, transferred to 10-mL ampoules, subjected to heating at 121°C for 15 min, and subsequently assayed by HPLC. The identity of diphenylmercury was confirmed by gas chromatography by autoclaving 5 mL of drops in a 10-mL ampoule and extracting the compound into diethyl ether (1·5 mL) by repeated aspiration. The ether extract was then submitted to gas chromatographic investigation.

Equilibration of drops at 50°C

To 20-mL glass tubes with screw-cap and PTFE wads pre-equilibrated to 50°C was added 10% sulphacetamide drops (5 mL) and at regular intervals the tubes were cooled on ice and immediately submitted to analysis.

To glass tubes with screw-caps and PTFE wads was added 10% sulphacetamide drops (5 mL) containing no phenylmercuric nitrate but with $HgCl_2$ (10^{-5} M) and the tubes were equilibrated to 50°C. To the tubes was added 50 μL of a solution of diphenylmercury (10^{-3} M) in acetonitrile to give a solution containing 10^{-5} M diphenylmercury. At regular intervals the tubes were cooled on ice and immediately submitted to analysis.

Results and Discussion

In the previous study of the stability of phenylmercuric nitrate in sulphacetamide drops, the organomercurial was assayed by HPLC of a dithiocarbamate complex by simple addition of an acetonitrile solution of the complexing agent to the drops (Parkin & Marshall 1990), the high concentration of sulphacetamide present eluted at the void-volume with considerable tailing making identification of the degradation products difficult (Parkin & Marshall 1990). The addition of a saturated solution of sodium chloride together with acetonitrile containing an internal standard followed by an aqueous solution of the complexing agent results in salting out of the acetonitrile to form a two-phase system with a small upper phase (the phase volumes determined in a measuring cylinder were 1·8 and 11·2 mL). Further, the ionic sulphacetamide sodium remains substantially in the lower phase whereas the hydrophobic dithiocarbamate complexes and diphenylmercury concentrate in the upper layer (partition coefficients upper/lower for the compounds were found to be 0·115 for sulphacetamide determined by dilution and ultraviolet spectrophotometry and for the mercury compounds determined by HPLC analysis of both the upper and lower phases: diphenylmercury = 120; $Hg(PIDTC)_2$ = 77; phenylmercury PIDTC = 95 and for the internal standard 275). The method, therefore, substantially removes the interfering sulphacetamide while concentrating the organomercury compounds and provides a solution in acetonitrile suitable for direct injection onto the HPLC column. This approach has been used previously for pre-concentration of organometallic complexes before HPLC analysis (Mueller & Lovett 1987; Parkin et al 1992a).

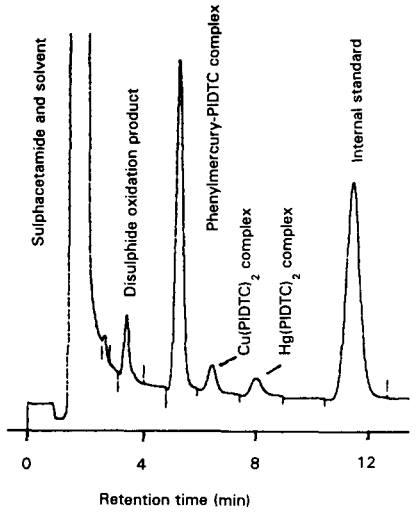

FIG. 1. Typical chromatogram of sulphacetamide drops (10%, w/v) after heating at 121°C for 15 min and assayed for phenylmercuric nitrate and Hg^{2+}.

Under the chromatographic conditions employed with monitoring at 258 nm, the PIDTC complexes of the phenylmercuric ion and Hg^{2+} elute well-resolved and separated from a small peak due to the copper complex (Fig. 1). The trace amounts of copper arise from contamination of the glassware used in the assay and do not interfere with the analytical method. The assay was validated for the concentrations of phenylmercuric ion and Hg^{2+} encountered in this study (Table 1). Diphenylmercury could not be assayed in the presence of the PIDTC reagent as the disulphide oxidation product arising from the slow atmospheric oxidation of the PIDTC reagent co-eluted. Diphenylmercury was therefore assayed by monitoring at the λ_{max} of diphenylmercury (226 nm) and substituting DEADTC for PIDTC. The oxidation product of this agent elutes at a shorter retention time than that of PIDTC and allows diphenylmercury to be quantified (Fig. 2). The assay was validated for the concentrations encountered in this study (Table 1). Performance of the assays on sulphacetamide drops prepared without phenylmercuric nitrate demonstrated that no other components interfered with the peaks due to phenylmercury PIDTC, $Hg(PIDTC)_2$, diphenylmercury or the internal standard.

Sulphacetamide drops of the APF were prepared with phenylmercuric nitrate being added last with analytical precision. Analysis of the drops showed no loss of phenylmercuric nitrate and no formation of either Hg^{2+} or diphenylmercury (Table 2). Following heat sterilization by autoclaving, substantial losses of phenylmercuric nitrate occurred with the formation of significant amounts of both Hg^{2+} and diphenylmercury. The identity of diphenylmercury was confirmed by comparison of the ultraviolet spectrum obtained by the use of a photodiode-array detector with that

Table 1. Statistical results for assays applied to mercury species.

Phenylmercuric nitrate	
Range:	0·1–2·4 × 10^{-3}% w/v (n = 8)
	(0·315–7·57 × 10^{-5} M)
Equation:	Peak area ratio phenylmercuric nitrate: internal standard = 0·9173 × concn (10^{-3})% w/v + 0·0026
Correlation coefficient:	0·9999
Coefficient of variation at 0·6 × 10^{-3}% w/v:	± 1·25% (n = 5)
Limits of detection*:	2 × 10^{-6}% w/v
Mercuric ion	
Range:	0·2–10 × 10^{-6} M (n = 7)
Equation:	Peak area ratio Hg^{2+}: internal standard = 0·03935 × concn (10^{-6})M − 0·00070
Correlation coefficient:	0·9999
Coefficient of variation at 2 × 10^{-6} M:	± 1·38% (n = 5)
Limits of detection*:	1 × 10^{-7} M
Diphenylmercury	
Range:	0·2–10 × 10^{-6} M (n = 7)
Equation:	Peak area ratio diphenylmercury: internal standard = 0·01498 × concn (10^{-6}) M + 0·00011
Correlation coefficient:	0·9998
Coefficient of variation at 1 × 10^{-6} M:	± 1·83% (n = 5)
Limits of detection*:	5 × 10^{-8} M

*Defined as the concentration at which the peak height is five times that of the average noise.

Table 2. Mean percentage of mercury species in sulphacetamide drops (n = 2, calculated as Hg^{2+}).

	Phenylmercuric nitrate	Hg^{2+}	Diphenylmercury	Total
Freshly prepared				
10% w/v	101·0	< 1	< 1	101·0
30% w/v	101·2	< 1	< 1	101·2
Following autoclaving at 121°C for 15 min				
10% w/v	77·5	5·4	9·3	92·2
30% w/v	71·5	9·3	12·5	93·3

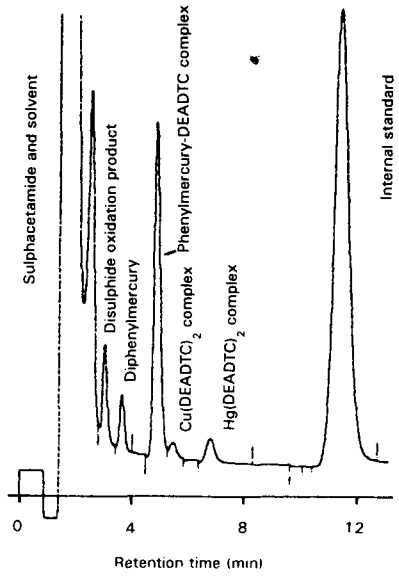

FIG. 2. Typical chromatogram of sulphacetamide drops (10%, w/v) after heating at 121°C for 15 min and assayed for diphenylmercury.

of an authentic sample obtained under identical conditions and by extraction with diethyl ether of the compound from a sample of the autoclaved drops and comparison of its retention time with that of an authentic sample by gas chromatography with mass detection of the most abundant ion (retention times: 21.36 and 21.39 min, respectively). Losses of phenylmercuric nitrate corresponded to those found in the previous study (20–30%) and most could be accounted for as Hg^{2+} and diphenylmercury. The rest may be accounted for by further degradation by pathways elucidated earlier (Parkin 1990; Parkin et al 1992a) or by adsorption to the ampoule.

The presence of both diphenylmercury and Hg^{2+} in the degraded drops suggested that these may be in equilibrium with the phenylmercuric ion (Fig. 3) and a study was undertaken under milder conditions (50°C) to assess whether this was so. When 10% w/v sulphacetamide drops were

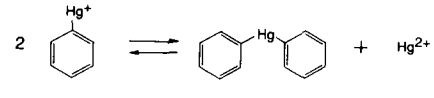

FIG. 3. Equilibrium formed between the phenylmercuric ion, diphenylmercury and Hg^{2+}.

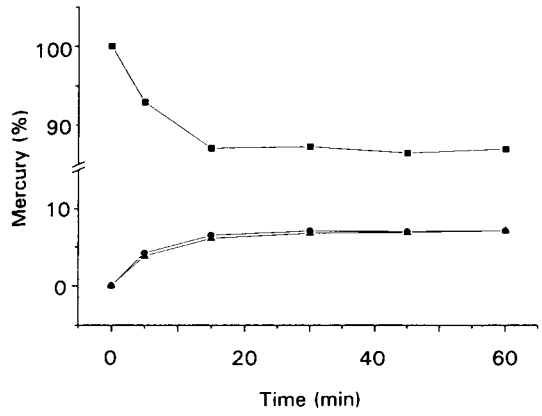

FIG. 4. Percent of Hg species in sulphacetamide drops (10% w/v) vs time when heated at 50°C. ■ Phenylmercuric ion, ● diphenylmercury and ▲ Hg^{2+}.

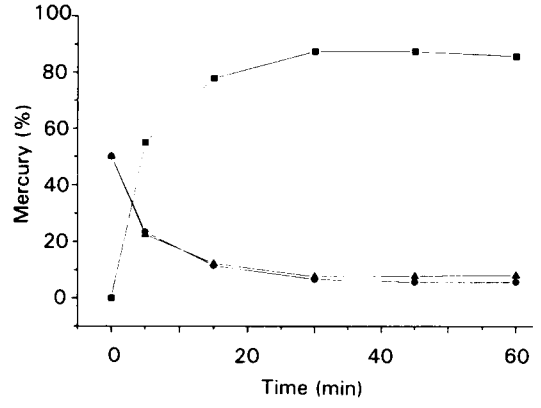

FIG. 5. Percent of Hg species in sulphacetamide drops (10%, w/v) containing 10^{-5} M diphenylmercury and Hg^{2+} vs time when heated at 50°C. ■ Phenylmercuric ion, ● diphenylmercury and ▲ Hg^{2+}.

heated at this lower temperature and analysed at regular time intervals it could be demonstrated that diphenylmercury and Hg^{2+} were rapidly formed in equimolar amounts with the loss of 13% phenylmercuric nitrate (Fig. 4). The quantities account fully for the original amount of phenylmercuric nitrate. To confirm these observations the experiment was repeated on drops in which $HgCl_2$ (10^{-5} M) was substituted for phenylmercuric nitrate and to which diphenylmercury was added at zero time to give a concentration of 10^{-5} M. The same equilibrium position was rapidly achieved (Fig. 5). The formation of diphenylmercury by the reductive process involving sodium metabisulphite is unlikely in this system as such a reaction involves the formation of metallic mercury rather than Hg^{2+} (Parkin 1990).

Equilibria of this type have been noted previously, in which arylmercury salts are converted to the corresponding diarylmercury compounds, the reactions proceeding in alkaline solution in the presence of chelating agents such as EDTA together with simple amines (Halpern & Garti 1975). The sulphacetamide possesses an aromatic amino group and this may serve as the auxiliary ligand together with EDTA to precipitate the establishment of this equilibrium. Under the more extreme conditions of heat sterilization, subsequent loss of Hg^{2+} by undefined mechanisms accounts for the inequality of the diphenylmercury and Hg^{2+} in the drops.

The antibacterial significance of the presence of this equilibrium for phenylmercuric nitrate in sulphacetamide drops is uncertain, as Hg^{2+} has antibacterial properties and the diphenylmercury is theoretically available via the equilibrium as phenylmercuric ion. Richards & McBride (1973) have studied the effect of various antibacterial combinations on the sterilization time of sulphacetamide drops following inoculation with *Pseudomonas aeruginosa* and found that the combination of phenylmercuric nitrate, EDTA and sodium metabisulphite was the least effective of the combinations studied. This reaction may partly explain these observations. The absolute loss of mercury is less than 10% when the drops are heat sterilized under the recommended conditions.

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