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Note

Assay of phenylmercuric acetate and nitrate in pharmaceutical products by high-performance liquid chromatography with indirect photometric detection

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Phenylmercury salts are widely used in pharmaceutical products as bactericides at concentrations of 0.001–0.002% (w/v)[1]. Their assay in these products may be performed by colorimetric methods[2–4], atomic absorption spectrometry[5–7], polarography[8,9], potentiometric titration[10], neutron activation analysis[11] or high-performance liquid chromatography (HPLC)[12,13]. The HPLC methods, to obtain the requisite sensitivity, involve either an extraction and concentration step prior to injection[12] or monitoring at 210 nm[13] which presumably must limit the applicability of the method.

Recently, widespread use has been made of indirect photometric methods for the detection and quantitation of poorly-absorbing or non-UV-absorbing compounds eluting from HPLC columns[14–20]. Methylmercury and phenylmercury form complexes with a variety of organic and inorganic ligands, those formed with thiol containing compounds having high association constants[21,22]. The adduct formed between phenylmercury (PM) and 6-mercaptopurine (6-MP) has been used for extraction of the latter from biological samples[23,24].

This paper reports the development of an HPLC method which utilises the phenylmercury–6-mercaptopurine complex (PM–6-MP) to provide a sensitive and specific method for the analysis of PM salts in pharmaceutical products.

EXPERIMENTAL

Reagents and chemicals

Phenylmercuric acetate and nitrate were obtained from BDH (Poole, U.K.) and the 6-mercaptopurine from Aldrich (Milwaukee, WI, U.S.A.). The methanol and acetonitrile were HPLC grade (Mallinckrodt, Melbourne, Australia).

Chromatographic equipment

The liquid chromatograph consisted of a pump (6000A, Waters Assoc., Milford, MA, U.S.A.), 20-μl loop injector (Rheodyne 7125, Cotati, CA, U.S.A.), variable-wavelength detector (LC-3, Pye-Unicam, Cambridge, U.K.), integrating recorder (Hewlett-Packard 3380A, Palo Alto, CA, U.S.A.) and a μBondapak C_{18} column (30 cm × 6.4 mm I.D., 10 μm particle size) (Waters Assoc., Sydney, Australia).

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Chromatographic conditions

The mobile phase consisted of methanol–acetonitrile–0.005 *M* KH_2PO_4 in water (1:4:5) containing $5 \cdot 10^{-4}$% (w/v) 6-mercaptopurine monohydrate.

RESULTS AND DISCUSSION

The PM–6-MP adduct has spectral characteristics such that it can be quantitated by monitoring at 293 nm (Fig. 1). At this wavelength the $5 \cdot 10^{-4}$% 6-MP in the eluting solvent results in a background absorbance of approximately 0.1 which results in a satisfactory baseline. Injection of an aqueous solution of PM acetate resulted in perturbation of the 6-MP concentration resulting in a negative peak at the retention time of 6-MP (immediately after the void volume) and the formation of a peak arising from the PM–6-MP adduct at 6.4 min (Fig. 2). The adduct is unstable under the chromatographic conditions employed and the injection of a solution of the PM–6-MP adduct using chromatographic solvent without 6-MP as mobile phase results in a peak due to 6-MP at 2.5 min and a small variable peak at 6.4 min. The presence of 6-MP in the mobile phase ensures that the PM–6-MP complex does not dissociate during chromatography.

The assay afforded a linear response over the range $0–5 \cdot 10^{-3}$% of PM acetate: area response = $618.9 \cdot 10^6$ [conc. % (w/v)] + $28.6 \cdot 10^3$ ($n = 5$, $r = 0.9998$). The coefficient of variation based on six replicate determinations of an 0.002% (w/v) solution of PM acetate was found to be 0.7%. Indentical results are obtained with PM nitrate and the calibration graph can be used for either bactericide provided correction is made for their relative molecular weights. The limit of detection (defined as peak height/noise = 3) of the assay is $1 \cdot 10^{-5}$%. Mercuric ion (Hg^{II}) can also be

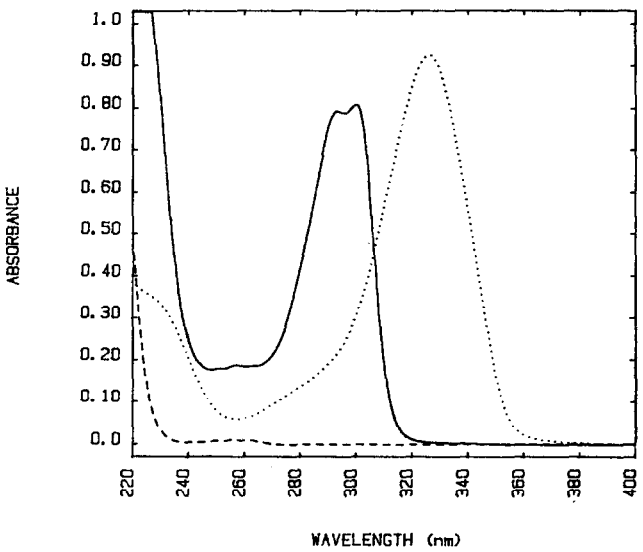

Fig. 1. Spectra of phenylmercuric acetate ($6.0 \cdot 10^{-5}$ *M*) (---------), 6-mercaptopurine ($5.9 \cdot 10^{-5}$ *M*) (.....) and the phenylmercury–6-mercaptopurine adduct made by mixing phenylmercuric acetate ($6.0 \cdot 10^{-5}$ *M*) with 6-mercaptopurine ($5.9 \cdot 10^{-5}$ *M*) (———). Solvent, 40% acetonitrile in 0.005 *M* KH_2PO_4.

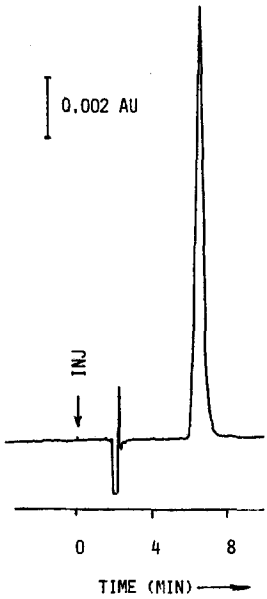

Fig. 2. Chromatogram of 0.002% (w/v) phenylmercuric acetate (20 μl). Solvent, methanol–acetonitrile–0.005 *M* KH_2PO_4 (1:4:5) containing $5 \cdot 10^{-4}$% (w/v) 6-mercaptopurine; flow-rate, 1.5 ml/min; monitoring wavelength, 293 nm. The peak at 6.4 min is due to the phenylmercury–6-mercaptopurine adduct.

detected by this system eluting (presumably as the dimercaptide adduct) at approximately 3 min. The assay can therefore quantitate both PM acetate and nitrate in the presence of free mercuric ions.

The stability of the adduct under column conditions has been evaluated by the use of stop-flow methods. Retention on the column for a period of 4 hr showed no loss of peak area demonstrating that the PM-adduct was unreactive to the metal column walls and was chemically stable.

To assess the scope of the method it was used to quantitate PM salts in pharmaceutical eye drops the formulae of which were derived from the *Australian Pharmaceutical Formulary and Handbook*[25] (Table I). In these cases all other UV absorb-

TABLE I

ANALYTICAL RESULTS OBTAINED FROM THE ANALYSIS OF EYE DROPS OF THE *AUSTRALIAN PHARMACEUTICAL FORMULARY*

Product	*Nominal content PM nitrate (%)*	*Found (%)*
Neomycin eye drops	0.002	0.00192
Fluorescein eye drops	0.004	0.00403
Chloramphenicol eye drops*	0.002	0.00195

* A commercially available brand of these drops showed minor peaks which co-eluted with the PM–6-MP adduct. Freshly prepared drops could be assayed by this method.

ing components in the formulations eluted prior to the PM–6-MP and did not interfere with the assay. The method would appear to have broad general utility and be suitable for the assay of phenylmercury salts in a range of pharmaceutical systems.

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