THE ANALYSIS OF ATROPINE SULPHATE AND ITS DEGRADATION PRODUCTS BY REVERSED-PHASE HIGH PRESSURE LIQUID CHROMATOGRAPHY

A.F. Fell, S.J.K. Johnstone, G. Smith, Department of Pharmacy, Heriot-Watt University, 79 Grassmarket, Edinburgh EH1 2HJ

The assay of atropine and related alkaloids is susceptible to interference from degradation products and, in dosage forms, from the formulation matrix. Chromatographic assays for tropane alkaloids by thin-layer (Mair & Smith 1977), gas-liquid (Liebisch et al 1973) or high pressure liquid chromatography (Brown and Sleeman 1978; Lund & Hansen 1978; Kreilgård 1978) have either lacked sensitivity and specificity, or have not been evaluated for quality control of dosage forms. The present work describes the development, quantitative evaluation and application of a rapid stability-indicating HPLC method for both routine quality control and stability studies of atropine sulphate in dosage forms.

The separation is based on 5 μm ODS-Hypersil in a 100 x 5 mm column, the eluent being acetonitrile/aqueous tetrabutylammonium sulphate (TBAS; 10 mM) and sodium acetate (50 mM) at pH 5.50 (25:75 % v/v). The phase capacity ratio (k') for atropine sulphate was 0.3, well-resolved from the major degradants tropic acid (0.9), atropic acid (1.7), apoatropine (2.7) and the internal standard 4-methylbenzoic acid (4.4); at a flow-rate of 1.0 ml min^{-1} the analysis time was 6 min. The degradant tropine was not detected at the UV-detector wavelength (254 nm). Benzalkonium chloride and phenylmercuric nitrate did not interfere.

Each eluent parameter was optimised in turn for all five components. Progressive increases in acetonitrile concentration from 10 to 30% led to reduced k' values and decreased resolution; at 25% the resolution was good and corresponded with maximum chromatographic efficiency ($N > 40,000$ plates m^{-1}). The optimum pH was 5.50. Increases in concentration of cationic pairing-ion TBAS from 5 to 20 mM gave linear increments in k' for the three acidic components, while increasing the acetate anion concentration from 25 to 100 mM led to a linear rise in k' for both atropine and apoatropine. This chromatographic behaviour indicates two ion-pair reversed-phase partition mechanisms (Tomlinson et al 1978).

Atropine sulphate eye drops B.P. (1%) and atropine sulphate injection B.P. (400 μg ml^{-1}) were assayed by HPLC after incorporation of internal standard at 80 μg ml^{-1}. Peak height ratios for atropine sulphate were linear with concentration up to 3.0 mg ml^{-1} and passed through the origin. At the test dilution level the 95% confidence limits (n = 12) were 2.50 $\pm$ 0.01 mg ml^{-1}. The relative standard deviation (RSD) for a batch of the eye drops was 2.1% (n = 6), recovery being 103.8% relative to label strength. For injections the 95% confidence limits (n = 12) were 432.5 $\pm$ 14.3 μg ml^{-1} and recovery 108.1%. Preliminary stability studies on atropine sulphate (2.4 mg ml^{-1}) heated at 100° in 0.2M NaOH indicated that after 4 minutes the extent of degradation (on a molar basis) was 60%, the principal products being atropic acid (54%) and apoatropine (5%). Similar treatment with 0.2M H_2SO_4 resulted in no detectable degradation.

This method has also been applied to the assay of homatropine hydrobromide eye drops B.P. (1% and 2%). Peak height ratios for homatropine hydrobromide (k' = 0.2) were linear with concentration up to 4.0 mg ml^{-1}. The 95% confidence limits at the test dilution level were 2.50 $\pm$ 0.05 mg ml^{-1} (n = 12). Recoveries were, respectively, 104.3% and 102.9%, RSD being 1.47% and 1.20%, for the 1% and 2% eye drops. This flexible and rapid procedure may also be used for the control of hyoscine hydrobromide in pharmaceutical preparations.

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