ANALYSIS OF METHYLXANTHINES BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

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The assay of methylxanthines in polypharmaceutical formulations is complex and may require preliminary separation. Analysis by uv-spectroscopy of preparations containing a single methylxanthine may be subject to spectral interferences by the formulation matrix. High pressure liquid chromatographic separation and quantitation by uv-detector offers high selectivity, speed and precision for routine quality control of dosage forms containing caffeine, theophylline, aminophylline or theobromine.

A reverse phase separation on 5μm ODS-Hypersil in a 100 x 5 mm stainless steel column has been developed using methanol/aqueous KH_2PO_4 (20:80) buffered to pH 6.00 as eluent at 1.5 ml min^{-1}. UV-detection with an 8 μl flow-cell was at 273 nm. The effect of progressive increases in methanol/buffer ratios from 10:90 to 60:40 (% v/v) at constant pH 6.00 was to reduce methylxanthine retention and decrease separation. Increases in pH at constant methanol/buffer ratio (20:80) exerted a profound influence on the retention of theoclate (8-chlorotheophylline), whose capacity factor (k') fell from 6.6 at pH 5.00 to 2.4 at pH 6.50. The retention of theobromine, theophylline, aminophylline and caffeine was unaffected by these pH changes. At pH 6.00 theoclate eluted between theophylline and caffeine with excellent resolution. Optimum eluent conditions for separation of the methylxanthines at room temperature were as specified above, when k' values were: theobromine 1.1; theophylline and aminophylline 2.4; theoclate 3.2; caffeine 4.6.

Theobromine and theoclate were considered as potential internal standards for the assay of preparations containing theophylline or caffeine. However, the relatively low aqueous solubility of theobromine and the pH-sensitivity and chromatographic variability of theoclate suggested the use of caffeine as internal standard at 500 μg ml^{-1} for Aminophylline Injection BP, Aminophylline Tablets BP and Franol tablets (120 mg theophylline, 8 mg phenobarbitone and 11 mg ephedrine hydrochloride). The regression line for peak area ratios of theophylline:caffeine was linear up to 750 μg ml^{-1} theophylline and passed through the origin. 95% confidence limits for theophylline in Aminophylline Injection BP were 2.06 ± 0.014 % w/v ($n < 10$). The coefficient of variation (CV) for a batch of the injections was 0.45% (n=8), recovery being 101.9% relative to label strength. Aminophylline Tablets BP were crushed, shaken with distilled water, the internal standard incorporated and the suspension filtered. The CV for theophylline content was 0.48% (n=4), recovery being 102.3%. Franol tablets were assayed similarly, CV being 1.53% (n=4) and recovery 98.8%. Elution from the column was complete in 5 minutes. Neither phenobarbitone nor ephedrine hydrochloride were detectable at the dilution level employed.

Taumasthman tablets, combining theophylline (100 mg) and caffeine (50 mg) with phenazone (100 mg), ephedrine hydrochloride (10 mg) and atropine sulphate (0.3 mg) were assayed similarly but using theobromine as internal standard at 100 μg ml^{-1}. The calibration graphs of peak area ratios for theophylline and for caffeine were linear and passed through the origin. 95% confidence limits ($n < 10$) for theophylline were 103.2 ± 0.3 mg and for caffeine were 49.9 ± 0.6 mg. Under these conditions the k' for phenazone was 8.0. Although well resolved from other peaks, the phenazone peak was broad and 95% confidence limits for the linear calibration graph were 105.2 ± 8.1 mg at the dilution level employed. Elution was complete in 8 minutes and neither ephedrine nor atropine interfered in the assay. This flexible and rapid procedure may also be used for the control of theobromine and caffeine in pharmaceutical preparations.