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Determination of caffeine and potassium sorbate in a neonatal oral solution by HPLC

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Summary

A reversed-phase HPLC method was developed and validated for the determination of caffeine and potassium sorbate in a neonatal oral solution. Chromatography was performed with a 100 × 4.5 mm Spherisorb 5 μm hexyl column with a mobile phase of 12% acetonitrile in 0.1 M sodium acetate buffer pH 4.5 and ultraviolet detection at 258 nm. The method is stability indicating and there was no interference from a number of common formulation ingredients, although benzoic acid did interfere.

Caffeine is used as a respiratory stimulant in premature neonates (Brouard et al., 1985). A hospital-produced formulation for oral use is available, containing caffeine citrate (10 mg/ml), preserved with potassium sorbate (1 mg/ml).

A number of authors have reported HPLC methods for the simultaneous determination of caffeine and sorbate (Matsunaga et al., 1986; Williams, 1986; Han et al., 1987; Bartko and Knesel, 1988; Ren and Hong, 1989). None of these methods were validated for used in pharmaceutical analysis. Also, the stability-indicating capability of these methods was not evaluated for caffeine or sorbate. The aim of the work described here was to develop and validate a stability-indicating HPLC method for the determination of caffeine and potassium sorbate in a pharmaceutical formulation for oral use.

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The ingredients of the caffeine formulation were of the appropriate pharmaceutical grade. Orcinol monohydrate was from Sigma Chemical Co. (Poole, Dorset, U.K.). All other chemicals and solvents were of analytical or HPLC grade. The HPLC system consisted of a Cecil CE1100 pump (Cecil Instruments, Cambridge, U.K.), Pye LC3 detector (Pye Unicam, Cambridge, U.K.) and a Shimadzu C-R3A integrator (Dyson Instruments, Houghton-le-Spring, Tyne and Wear, U.K.). Samples were injected by a Talbot ASI-4 autosampler equipped with a Rheodyne 7010 injection valve (Talbot Instruments, Alderley Edge, Cheshire, U.K.).

The column was a 100 × 4.5 mm Spherisorb 5 μm hexyl reversed-phase column (Hichrom, Reading, U.K.). The mobile phase was 12% ace-

tonitrile in 0.1 M sodium acetate buffer pH 4.5, at a flow rate of 2 ml/min. The injection volume was 20 μl and the detector wavelength was 258 nm.

Caffeine oral solution (1.0 g) was diluted to 25 ml with water. To 5 ml of this solution was added 5 ml of a 3 mg/ml aqueous solution of orcinol monohydrate (internal standard) and the mixture diluted to 50 ml with water. The weight per ml of the oral solution was determined according to the method of the British Pharmacopoeia (1988) each time analysis was performed, in order to convert the assay results to a weight in volume basis.

The standard was prepared by mixing 5 ml of a 3 mg/ml solution of orcinol monohydrate, 2 ml of a 1 mg/ml aqueous solution of caffeine citrate, and 2 ml of a 0.1 mg/ml aqueous solution of potassium sorbate. The mixture was diluted to 50 ml with water.

System suitability tests were performed each time the method was performed. The precision of chromatography was checked by chromatographing six replicates of a standard. The peak area ratios of both caffeine citrate/internal standard and potassium sorbate/internal standard should not be greater than 2% relative standard deviation (RSD). Values were typically in the range 0.3–0.8% RSD for both. The resolution factor, as defined by the British Pharmacopoeia (1988), should be not less than 1.0 for all peaks.

Samples and standards were injected in duplicate. Quantification was by peak area measurement.

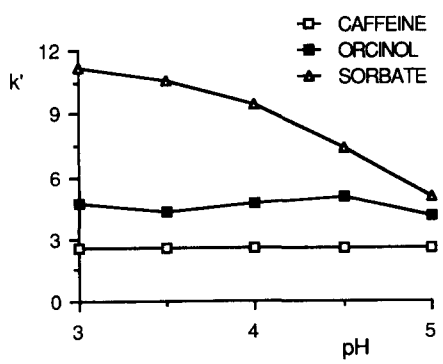

Fig. 1. Effect of mobile phase pH on capacity factor (k') for caffeine, orcinol and sorbate.

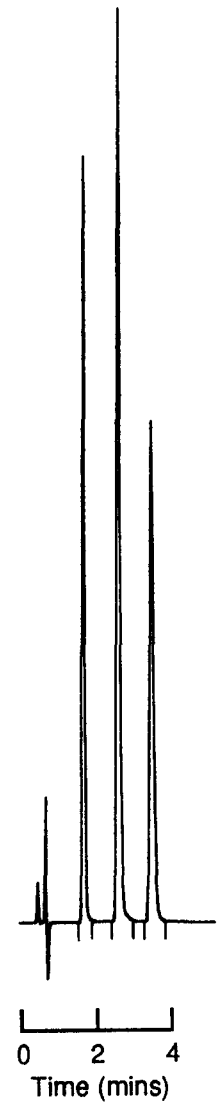

Fig. 2. Chromatogram of caffeine neonatal oral solution. Retention times: caffeine, 1.7 min; orcinol (internal standard) 2.6 min; sorbate 3.5 min.

Preliminary studies used a Spherisorb ODS column; however, a hexyl column was adopted because this gave more symmetrical peaks. Optimal separation of caffeine and orcinol was achieved with a mobile phase having 12% acetonitrile. A buffer pH of 4.5 gave optimum separation of orcinol and potassium sorbate (Fig. 1).

The wavelength of maximum absorbance of potassium sorbate in the mobile phase (258 nm)

was chosen as the analytical wavelength in order to obtain similarly sized chromatographic peaks for caffeine and sorbate (Fig. 2).

Sampling of the oral solution was by weight rather than volume because its slightly viscous nature led to poor reproducibility if it was pipetted without prior dilution.

There was a linear relationship between peak area and concentration for orcinol monohydrate (1.5–12 μg injected), caffeine citrate (0.2–1.6 μg injected) and potassium sorbate (0.02–0.16 μg injected). This corresponds to 25–200% of the concentrations specified in the method. In all three cases, the correlation coefficient was 0.9999 and the regression passed through the origin.

An oral solution prepared with accurately known quantities of caffeine citrate and potassium sorbate was assayed six times on the same day, using duplicate injections for each separate assay and a single standard. Mean recoveries were within 1% of the expected values for both components, using either the internal or external standard methods of calculation. The 95% confidence limits were $\pm 1.5\%$ for both components with the internal standard method and $\pm 1.1\%$ for both components with the external standard method of calculation.

The stability-indicating capability of the method was assessed by refluxing the oral solution with either sodium hydroxide (0.1 M) or sulphuric acid (0.05 M) for 1 h. After cooling, the pH was adjusted to neutral with acetic acid or 2 M sodium hydroxide. Some unresolved peaks eluting close to the void volume were observed with the alkali-treated sample. None of these peaks caused interference. No additional peaks were seen with the acid-treated sample.

The suitability of the method for use in the presence of oxidative degradation was assessed by chromatographing samples of the oral solution which had been stored in the dark at 4 and 32°C (± 1°C) in partially filled containers for up to 4 years. In both samples, less than 1% of the original concentration of potassium sorbate remained. Additional peaks, seen only with high detector sensitivity, were observed eluting close to the void volume, but no interfering peaks were observed.

The applicability of the method to other pharmaceutical formulations was assessed by chromatographing selected pharmaceutical additives at concentrations similar to those expected in formulations. There was no interference from amaranth, ascorbic and citric acids, disodium edetate, saccharin sodium, sodium acetate, sodium metabisulphite, tartrazine or vanillin. Methyl, ethyl and propyl-4-hydroxybenzoates did not interfere with chromatography but could present a problem because of their extended elution times ($k' = 13.2$ for the methyl ester). Benzoic acid was not separated from potassium sorbate.

The results indicate that the method is linear, precise, accurate and stability-indicating. In HPLC systems capable of injecting reproducible volumes, the internal standard could be omitted. Its inclusion would, however, allow the method to be used in situations where less reproducible injection volumes could be achieved. The method is rapid, with chromatography requiring approx. 4 min.

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