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Micellar electrokinetic chromatography stability indicating assay and content uniformity determination for a cholesterol-lowering drug product

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Abstract

This study describes a specific, linear, precise, accurate and sensitive method for the determination of a developmental cholesterol-lowering drug formulated in capsules. The method can also determine two known hydrolytic degradants of the drug. Samples are dissolved in acetonitrile–phosphate buffer pH 4.5, diluted with water and assayed by micellar electrokinetic chromatography (MEKC) in a buffer containing 0.1 M borate–0.025 M SDS at 30°C with an applied voltage of 25 kV. Detection is by UV absorbance at 200 nm. The method was cross validated by comparison with a gradient elution HPLC method. The MEKC method gave at least equivalent precision, accuracy and sensitivity to HPLC but was superior in the resolution of the known impurities and gave a considerably shorter analysis time. The method has been accepted as part of a regulatory submission to the US Food and Drug Administration (FDA). © 1997 Elsevier Science B.V.

Keywords: Micellar electrokinetic chromatography; Potency; Degradants; Content uniformity; BMS-188 494

1. Introduction

HPLC has for many years been the method of choice for the analysis of pharmaceutical drug substances and products. Ideally an isocratic elution of the components will be used, however when an analysis is required for both the drug and its degradation products, the analytes may be of such a differing polarity that gradient elution conditions are required for the separation. Capillary electrophoresis and its derivative forms such as micellar electrokinetic chromatography (MEKC) offer an alternate selectivity to HPLC, enabling compounds with differing polarity to be separated using 'isocratic' conditions, which do not require -extensive column equilibration between analyses. These principles have been demonstrated for the analysis of a cholesterol-lowering drug, BMS-188 494 (Fig. 1) and its ester hydrolysis degradation products BMS-187 745 and BMS-196 716.

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2. Experimental

2.1. Materials

Boric acid, sodium tetraborate, sodium hydroxide and orthophosphoric acid AnalaR grade, di-potassium hydrogen orthophosphate, HiPerSolv grade and sodium dodecyl sulphate (SDS) specially pure grade were all purchased from BDH, Poole, Dorset, UK. All drug compounds were obtained from the Bristol-Myers Squibb Pharmaceutical Research Institute. All water was purified by reverse osmosis to USP purified water grade.

2.2. Reagents

0.2 M borate buffer was prepared by dissolving 4.2 g of boric acid and 3 g of sodium tetraborate in 500 ml water. The electrophoretic run buffer was prepared by mixing 1:1 (v/v) 0.2 M borate buffer and 0.05 M SDS and filtered through a 0.45 μm membrane filter. 0.01 M phosphate buffer was prepared by dissolving 1.74 g of anhydrous di-potassium hydrogen orthophosphate in 1 l of water, having adjusted the pH to 4.5 using 10% (v/v) orthophosphoric acid. Sample diluent was acetonitrile–0.01 M potassium phosphate buffer pH 4.5, (1:1, v/v).

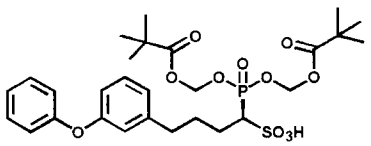
2.3. Apparatus

A Beckman P/ACE system 5510 fitted with a UV diode array detector (Beckman Instruments, High Wycombe, Bucks, UK) was used. A 57 cm × 75 μm i.d. (50 cm to detector) fused silica capillary was fitted in a cartridge with a 100 × 800 μm detection aperture. The new capillary was conditioned before use by rinsing with 1 M NaOH for 20 min, followed by 0.1 M NaOH for 10 min and run buffer for 10 min. A new capillary was equilibrated after conditioning, with run buffer (20 min at 25 kV). The applied voltage across the capillary was 25 kV and the capillary was maintained at a temperature of 30°C. Analytes were detected by UV absorbance at 200 nm. Between injections, the capillary was rinsed with 0.1 M NaOH for 0.5 min followed by run buffer for 1 min. Sample was introduced to the capillary at the anode, using 5 s pressure followed by 1 s run buffer. Sample and standard solutions were allowed to equilibrate in the autosampler tray for 30 min before injection.

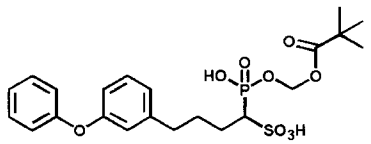
3. Results and discussion

3.1. Procedure

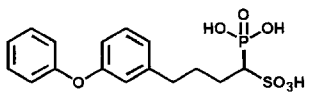
Capsule formulations were prepared at several potencies (5 and 50 mg) to evaluate the safety and efficacy of the drug. Stability studies were also performed on these formulations and hence the analytical potency methodology was required to indicate stability. The drug itself (BMS-188 494) was known to degrade to its mono ester (BMS-196 716) and also its free acid form (BMS-187 745). The polarity of the drug differed greatly from its degradation products, and all stability indicating HPLC methodologies developed required gradient elution, with a run time of 30 min to determine all three compounds from a single sample solution. The use of MEKC to perform the separation was investigated and using the



BMS-188494



BMS-196716



BMS-188745

Fig. 1. Structures of BMS-188 494, BMS-196 716 (monoester degradation product) and BMS-188 745 (free acid degradation product).

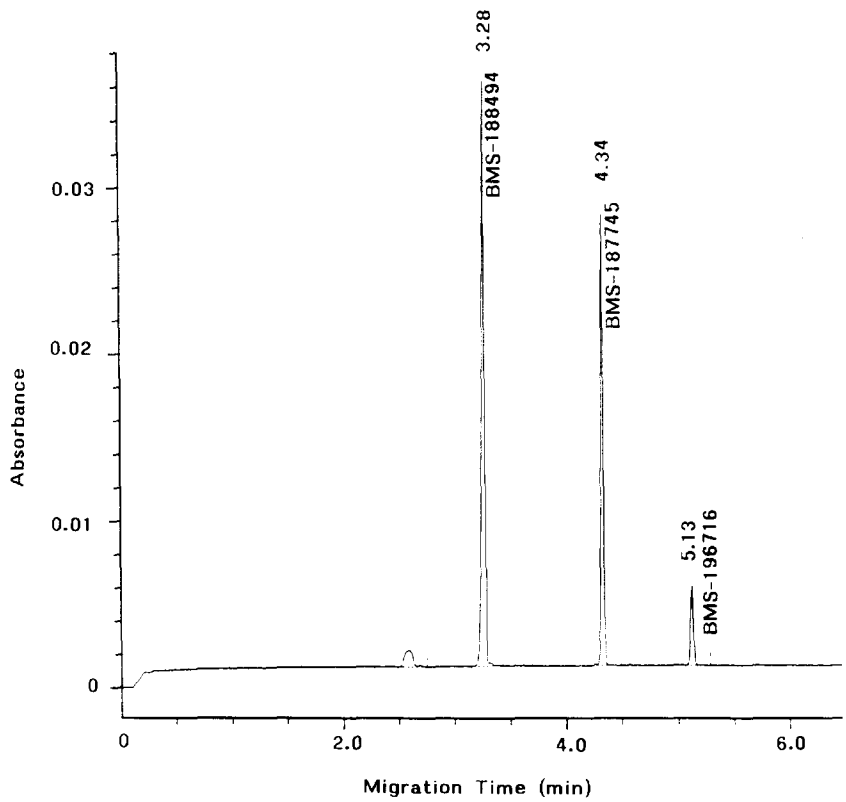


Fig. 2. Electropherogram of the separation of BMS-188 494, BMS-196 716 (monoester degradation product) and BMS-188 745 (free acid degradation product).

optimised conditions described in Section 2, separation of the drug from its degradation products could be obtained in 8 min. Samples were prepared by dissolving the capsule contents in acetonitrile–0.01 M phosphate buffer pH 4.5, 50:50 (v/v) to give a concentration of 0.5 mg ml^{-1} that was further diluted with water to give a working sample solution concentration of 0.1 mg ml^{-1}. Quantitation was achieved using peak area corrected for time, with an external standard prepared in the same diluent as the sample solution, at a final concentration of 0.1 mg ml^{-1}. Quantitation of degradants was made by peak normalisation. A system suitability test was established using a solution containing both BMS-188 494 and its free acid degradation product BMS-187 745 (the peak closest to BMS-188 494) at a concentration of 5%. A resolution ratio of > 17 was defined for the two compounds with an R.S.D. of $< 2\%$ for the BMS-188 494 peak area of three replicate injections.

3.2. Validation

The method has been validated using commonly applied method validation criteria [1–3]. The electrophoretic separation was selective for the drug (BMS-188 494) and its degradation products (Fig. 2). When solutions of capsule placebo blends and the sample diluent were injected, no peaks were seen in the electropherogram at the retention times of the drug or its known degradation products. The linearity of absorbance response of BMS-188 494 standard with respect to concentration was determined over the range equivalent to 0–150% of the nominal sample and standard solution concentration. The linearity was also determined for the two known degradation

products over a range equivalent to 0–20% of the nominal concentration of BMS-188 494 in the standard solution. Linear regression analysis was determined at the 95% confidence level and each analyte had a correlation coefficient of 0.999 with a y-intercept that was not significantly different from zero. The linear regression residual plot for each analyte confirmed the linearity of the response. Fig. 3 is given as an example for BMS-188 494. The precision of ten replicate determinations of BMS-188 494 was performed, making all replicate injections from the same sample vial. Typical R.S.D. values of 0.5% were obtained (range 0.4–0.8 over three days). By comparison, HPLC of the same sample solutions, using gradient elution conditions gave typical R.S.D. values of 0.8%. The accuracy of the method was determined by spiking placebo capsule contents, with accurately weighed quantities of BMS-188 494 at the nominal concentration and the known degradation products at a level of 2.5% of the nominal BMS-188 494 concentration. The analyte concentration was then determined by MEKC and HPLC and the percentage recovery determined (Table 1). The accuracy of the determination of BMS-188494 and its known degradants by MEKC is acceptable and is confirmed by comparable recoveries obtained using HPLC analysis. The limit of detection and quantitation for the known degradants was 0.05 and 0.1% of the BMS-188 494 concentration as determined by dilution of standard solutions of known concentration. These limits of detection are similar to

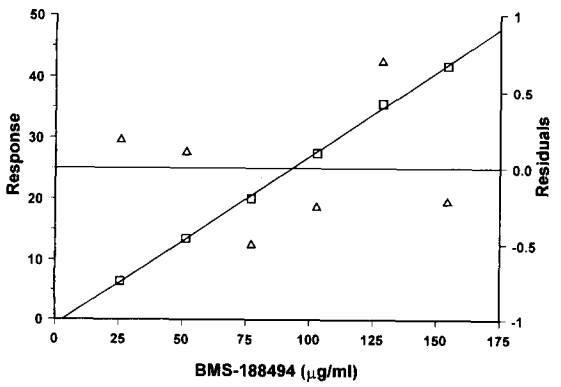


Fig. 3. Linearity of response (□) and residual plot (△) for BMS-188 494.

Table 1
Recovery of BMS-188 494 and its known degradation products by MEKC and HPLC

Analyte	MEKC (%)	HPLC (%)
BMS-188 494	100.3	99.5
BMS-196 716	103.7	103.4
BMS-187 745	99.9	99.9

those observed for the analysis of other cholesterol lowering drugs using MEKC [4]. The electropherogram shown in Fig. 4 demonstrates the separation of BMS-188494 from its two known degradants in a sample of the drug substance which had been stored at 40°C and 75% RH for three months.

4. Conclusion

The application of MEKC to the analysis of BMS-188 494 demonstrates many of the advantages of the technique. This application is particularly simple in its operation compared to the alternate HPLC gradient analysis. Electrophoresis is performed on a simple and inexpensive fused silica capillary that can be used for many other analyses. The use of an internal standard is often recommended in capillary electrophoresis to improve the analytical precision, which is sometimes attributed to injection volume fluctuations. [3,5,6]. Occasionally, precision using an external standard may be better than using an internal standard [7]. In the method reported, excellent precision, at least as good as the HPLC procedure was, has been demonstrated without using an internal standard. This may reflect the development of better injection systems on newer instruments as well as a better understanding of injector operating procedures. The run time is only 8 min compared to the 30 min HPLC gradient analysis, and unlike the HPLC system there is almost no equilibration of the capillary between analyses. Not only were run times reduced but the time for development and validation was also reduced. CE is less sensitive when directly compared to HPLC [8]. However, by using a detection wavelength of 200 nm, which is below the UV cutoff for HPLC solvents, the sensitivity of the MEKC determination is

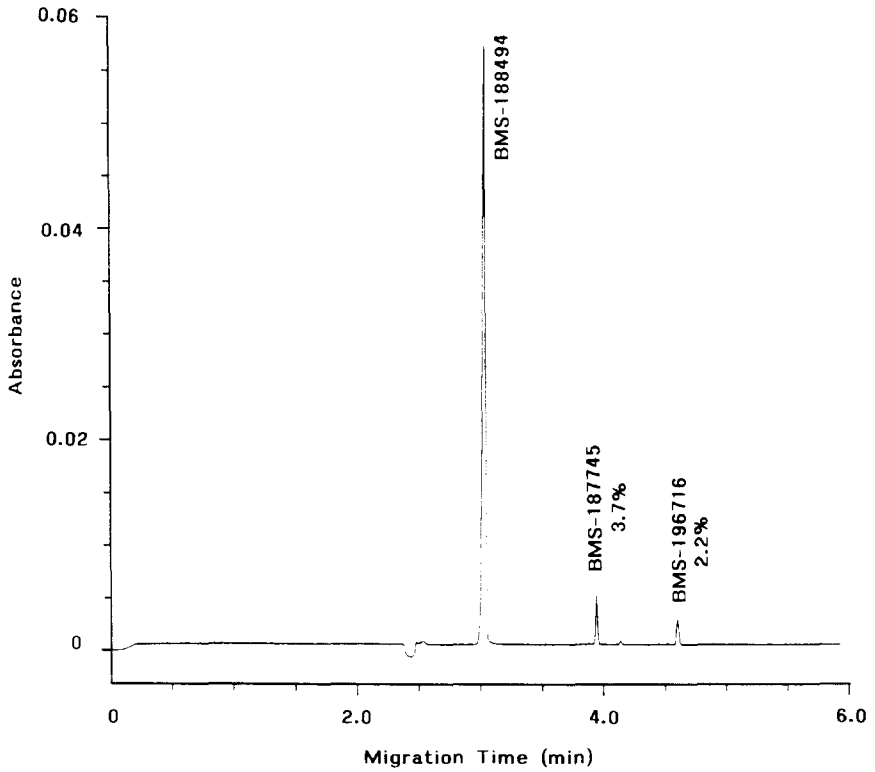


Fig. 4. Electropherogram of the separation of BMS-188494 from its degradant impurities, BMS-196716 (2.2%) and BMS-188745 (3.7%) from a sample of the drug substance which had been stored at 40°C and 75% RH for three months.

equivalent to that obtained by HPLC operated at the lowest practical UV wavelength of the analytes (215 nm), enabling the method to be used to analyse degradation products at an appropriate level. The suitability of the application for use as a stability indicating assay and content uniformity procedure have been endorsed by its acceptance in a regulatory submission to the US Food and Drug Administration (FDA).

Acknowledgements

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