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Rapid resolution of drugs and related substances with an eCAP™ polyamine coated capillary

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Abstract

The long term stability of a commercial polyamine coated capillary (eCAP™) is described. The capillary, which can be used in the CZE and MEKC mode, is based on coating with a polyamine after conditioning with 1 M NaOH and regeneration of this coating after each run. The stability was tested over 6 months on the drug trimethoprim and the R.S.D. values for migration time and peak area were 2.86 and 3.62% respectively ($n = 8$, each time of determination) (> 600 sample injections over the period). This stability was utilised in the validated method developed for trimethoprim and four of its related impurities.The repeatability of peak area for trimethoprim (without normalisation or external standard) was, within-day R.S.D. = 1.02% ($n = 8$) and between-days R.S.D. = 2.02% ($n = 8$ each day). Linearity was good (for 50 μg ml^{-1} target) ($y = 249.6x + 17.3$ ($r = 0.992$, $n = 6$). These results for trimethoprim and for other drug mixtures were comparison with conventional capillaries and the advantage of reducing the polyamine treated eCAP™ capillary to a minimum length is described, to achieve rapid assay of the 5 component timethoprim mixture in < 2 min. © 1997 Published by Elsevier Science B.V.

Keywords: Trimethoprim and related substances; Drug assays; Polyamine coated capillary; Long-term capillary stability; Method development and validation; Capillary electrophoresis

1. Introduction

As in RP-HPLC packings, the surface charge on a fused silica capillary used in capillary zone electrophoresis (CZE) can cause difficulties in the assay of a basic drug. Dependant upon pH, a high electroosmotic flow velocity (V_{eo}) and negative surface charge can give poor resolution through combinations of rapid passage across the capillary and interaction with the silica surface. A number of solutions to these problems have been studied. These include separation at low pH to reduce the electroosmotic flow (EOF) to a minimum [1], introduction of additives to the electrolyte such as tetradecyltrimethylammonium bromide (TTAB) to manipulate the EOF [2] and dynamic coating of the silica wall with short alkyl celluloses (e.g. methyl cellulose [3]), changing the CE mode and the more permanent coating of the silica walls to remove the high negative charge [4].

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This latter mode of coated capillaries has been used in CZE analysis of biological samples to increased separation efficiency and resolution [5,6]. In addition positively charged capillary surfaces, such as an amino propyl trimethoxysilane (APS) bonded phase, has been developed for CE/mass spectrometry [7], in which a reversal in the EOF results from the surface treatment. Recently in commercial terms, a modified capillary of this type has been marketed. The eCAP™ (Beckman Instruments, UK) capillary is treated with a polyamine to give a strong cation surface to the capillary wall.

In this study the performance of this polyamine treated eCAP™ capillary has been examined with a number of basic drug mixtures and also drugs and their related substances which contain basic, acidic and neutral functionalities. In particular the stability of the capillary and the effects of change over a range of pH was examined. In addition to stability, the advantage of reducing the length of the coated capillary to an allowable minimum was also studied to illustrate how improved resolution on the polyamine coated capillary could be coupled with reductions in migration time, to obtain rapid resolution of a number of components in a minimal migration time window [8].

A review of the fundamental equations relating to CZE [9] would indicate that performance gains could be achieved by using shorter capillaries together with the possibility of lower operating voltage and increased buffer concentration to improve peak efficiency through sample stacking. This should lead to fast analysis times, with limitations on band broadening through molecular diffusion.

2. Experimental

2.1. Instrumentation

The CE system was either a: Beckman P/ACE System 2210 (Beckman Instruments, UK) controlled by an APS2, IBM PC through Beckman System Gold software, or an ABI Model 270A Capillary Electrophoresis system (Perkin Elmer/ABI, Warrington, UK).

2.2. Capillaries

The eCAP™ amine capillary was either 57, or cut to 37 or 27 cm length (50, 30 or 20 cm respectively to the detector) and 50 μm i.d. (Kindly supplied by Beckman Instruments, UK). The conventional fused silica capillaries were also of the above dimensions and obtained from Composite Metal Services (Worcs, UK).

2.2.1. Capillary conditioning

The standard procedure for the eCAP™ was to initially condition the treated capillary supplied by Beckman, and then to use the regenerator continuously:

(a) Flush capillary 5 min with operating buffer (here 50 mM sodium acetate pH 4.2).

(b) Flush with polyamine capillary regenerator (as supplied by Beckman) for 2 min.

(c) Inject the sample.

(d) Run buffer.

(e) After run, 1 M NaOH for 2 min.

(f) At the end of run, wash with regenerator 2 min and then buffer for 2 min (leaving in buffer overnight).

2.3. Drugs and related substances

The drug substances for the sample mixtures are shown in Fig. 1 and comprised of: (a) trimethoprim (TMP) and related substances (**1**–**5**); (b) acyclovir and guanine (**6**,**7**); (c) salbutamol, lamivudine, imidazole and aminobenzoic acid (**8**–**11**).

3. Results and discussion

3.1. Electroosmotic flow and robustness testing

In the initial experiments with TMP and its 4 related substances, comprising basic, acidic and neutral functionalities, an optimised method was developed on a conventional silica capillary (57 cm length), as shown in Fig. 2a. The resolution on the eCAP™ capillary of the 5 components in the mixture was then slightly adjusted from these conditions (including a change in polarity) to give

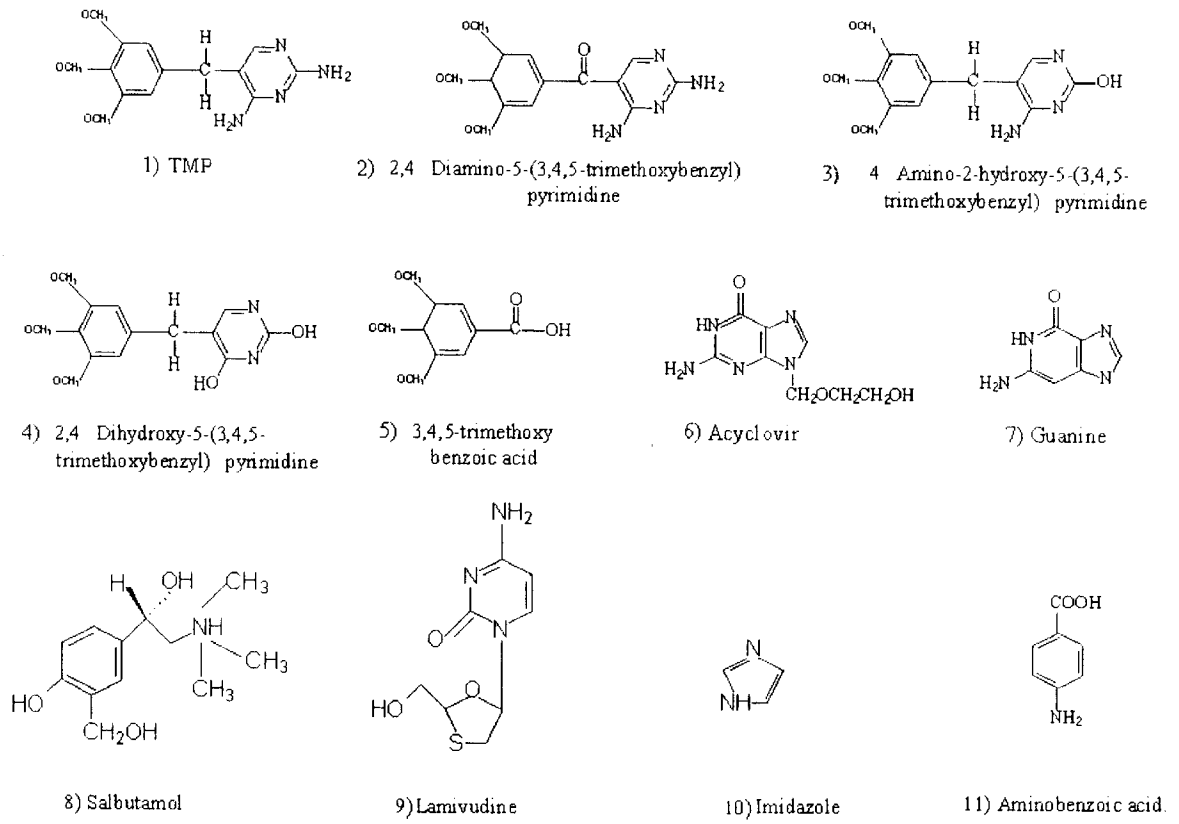

Fig. 1. Structure of the drugs and related substances contained in the drug mixtures **1**–**5** associated with TMP; **6**–**7** with acyclovir and **8**–**11** as a basic drug mixture.

Fig. 2b. In both cases resolution of all the components was observed, although on the conventional capillary components **2** and **3** just gave baseline separation and component **4** gave a broad peak.

In contrast, all 5 components were clearly resolved on the polyamine coated capillary in a shorter overall migration time and with higher peak efficiencies throughout. Interestingly these results were possible under nearly the same operating conditions of buffer, its pH, applied voltage and temperature conditions.

The choice of buffer is an interesting aspect when using this coated capillary. In the commercial kit sodium dihydrogen phosphate at pH 2.0 and 7.0 was provided. The phosphate is suggested to reduce the positive capillary surface charge by pairing the positive charge of the amine ions with the negative charge on the phosphate ions, to give a resultant reduction in the EOF [10]. However, although this effect has been shown to exist with phosphate buffers in other separations in our experimental work, acetate gave the best resolution and peak shape for the drug mixture examined.

Although the results were reasonably impressive, a crucial test of this approach is the overall stability of the assay during method validation and when used over an extended time period. In the case of many of the other procedures of treatment of the capillary surface, question marks have been raised against their long-term stability, when applied in 'real situations' such as batch assay of a pharmaceutical preparation. Therefore the first test of this separation procedure stability was to check the repeatability and reproducibility of the assay method through the normal statistical

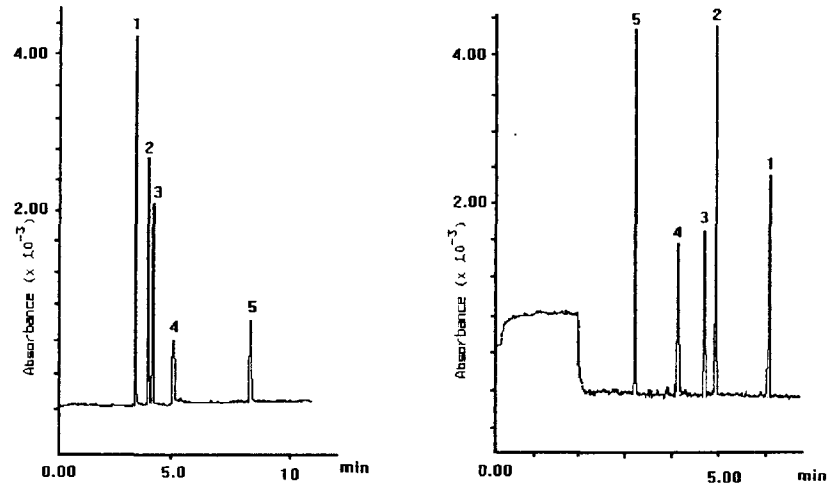

Fig. 2. The separation of TMP and its four related substances on: (a) a conventional silica capillary (70 cm (50 cm) × 50 μm i.d.) (ABI 270A instrument), and (b) the polyamine coated eCAP™ (57 cm (50 cm) × 50 μm i.d.), (Beckman P/ACE 2210) where the operating parameters for both were: buffer 50 mM sodium acetate (pH 4.2); voltage −20 kV; wavelength −254 nm; temperature −30°C. Key to the electrophoretic peaks: (1) TMP; (2) 2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine; (3) 4-amino-2-hydroxy-5-(3,4,5-trimethoxybenzyl) pyrimidine; (4) 2,4-dihydroxy-5-(3,4,5-trimethoxybenzyl) pyrimidine; (5) 3,4,5-trimethoxybenzoic acid.

parameter of within-day and between-days. The results on peak area from within-day were R.S.D. = 1.02% ($n = 8$) and between day (over 5 days) R.S.D. = 2.02% ($n = 8$ each day). During this work it was clear that the eCAP™ capillary was very stable within the period of weeks when this work was completed and this was further tested with a long-term study. For TMP, very impressive consistency of the results were shown, on comparison between the weekly results on the first and last day, July to December where >600 injections gave an R.S.D. = 2.86% on migration time and R.S.D. = 3.62% on peak area ($n = 8$ each time of analysis), for the same capillary used regularly over the six month period. Because of the high stability of the method, these results were calculated using the raw data, without peak area or migration time normalisation or external standardisation.

The capillary featured above (supplied by Beckman) was kept for the purpose of analysis of the TMP mixture, but it was used continuously during the 6 month time period where the pH of the capillary was varied regularly and in addition different batches of amine regenerator were used.

After these repeated operations, the optimised methods on both the conventional capillary and the polyamine coated capillary were compared as shown on Table 1.

3.2. Improvement in peak efficiency

To illustrate again the improvement in resolution and electrophoretic peak efficiency of the eCAP™ capillary, a two component basic drug mixture of acyclovir, an antiviral agent, and its major metabolite and degradation product, guanine, was tested (Table 2, Fig. 3) and a much improved peak efficiency was given over the conventional CZE method. This improvement results from the reduction in the wall interactions and from the stacking effect from the difference in buffer concentration, going from 20–50 mM and so accentuating the difference between the sample solution and the buffer conductivity. This change in the buffer and pH was due to the choice of optimised conditions on the coated capillary against the conventional capillary.

Table 1
Method validation parameters for TMP and the 4 related substances on the 57 cm × 50 μm i.d. capillaries with 50 mM sodium acetate buffer (pH 4.2) voltage 20 kV, temperature 30° and wavelength 254 nm

Statistical parameter	eCAP™ Capillary					Conventional Capillary				
Peak No.	1	2	3	4	5	1	2	3	4	5
Repeatability (R.S.D. %)										
Migration time (min)	0.94	0.98	1.11	1.21	1.03	0.31	0.32	0.38	0.51	1.40
Peak Area	1.15	0.92	1.21	0.89	1.26	1.32	1.45	1.65	1.34	1.29
Linearity ($n = 6$) (forTMP)	$y = 249.6x - 17.3$ ($r = 0.992$)					$y = 579.3x - 521.1$ ($r = 0.994$)				
Limit of Detection										
3 × Baseline noise	1 μg ml^{-1}					1 μg ml^{-1}				
Limit of quantitation, 3σ	5 μg ml^{-1}					5 μg ml^{-1}				

3.3. Stability of the eCAP™ capillary to pH changes

In some reported methods with coated capillaries a limited operating range of electrolyte pH is recommended and outside these ranges marked effects, including cleavage of the capillary coating, can result. However, with the eCAP™ capillary a wide range of operating pH was possible (pH 2–10). In this programme the pH was moved across the range a number of times during method development, but these changes had limited effects on assay performance as shown for one of the drug mixtures examined (Fig. 4). A check at the higher pH values is recommended, however, with acidic compounds for possible interaction with the coated polyamine phase.

One aspect of capillary wall stability suggested by the manufacturer is the recommendation that the capillary is washed with 1 M NaOH between runs, but should not be allowed to stand in NaOH for more than a few minutes. However, on examination of this by leaving the capillary in 1 M NaOH overnight, no detrimental effects on performance were shown after applying the standard amine solution regeneration step prior to assay. Although this action is obviously not recommended in the long term because of the deleterious effect on the capillary wall, it suggests that the length of time the NaOH is in the capillary is not as vital as initially suggested.

In addition, in the operating recommendations for the eCAP™, 1 M NaOH is suggested as the best washing solution between runs to obtain a reproducible capillary surface and, in this study, a comparison was carried out between 1 M NaOH versus 0.1 M NaOH (as used in conventional CZE assays). For a basic drug test mixture of aminobenzoic acid, lamivudine and imidazole on a 37 cm capillary and tris buffer at pH 8.0, the repeatability results for migration times were 1 M NaOH: R.S.D. = 0.50; 0.70, 0.84% ($n = 8$); and 0.1 M NaOH: R.S.D. = 0.83; 1.86, 1.77% ($n = 8$) respectively which, although not a major difference, is sufficient to suggest that better precision in migration time can be obtained from the higher concentration alkali wash.

3.4. Capillary length, applied voltage and analysis time

It was then intended to build upon the improvements in the overall analysis time and peak efficiency and shape obtained on the eCAP™ capillary, as discussed above, by further reductions in migration time with shortening of the capillary length from the conventional 57 cm (50 cm to detector) to 37 cm (30 cm to detector). The improved results for TMP and its related substances, prepared in MeOH–35 mM acetate buffer (pH 4.2) (15:85, v/v), are illustrated for the 37 cm eCAP™ capillary in Fig. 5b, where the total migration time is 2.34 min versus 6.02 min for the conventional 57 cm capillary (Fig. 5a).

Table 2
Repeatability of migration time and peak efficiency for acyclovir/guanine

R.S.D. %	Migration time		Peak efficiency	
	eCAP™	Conventional	eCAP™	Conventional
Acyclovir	0.26% ($n = 8$)	1.3%	1.9×10^5 (plates m^{-1})	2.5×10^4
Guanine	0.28% ($n = 8$)	1.3%	2.43×10^5 (plates m^{-1})	
Conditions	eCAP™	Conventional		
Capillary	57 cm (50 cm to detector) × 50 μm	72 cm (50 cm to detector) × 50 μm		
Buffer	50 mM acetate pH 4.2	20 mM citrate pH 2.5		
Voltage	30 kV	30 kV		
Temp	25°C	25°C		

Although a relatively large decrease in the migration time is shown, the effect has to be balanced by slight peak broadening which, although insufficient to considerably reduce the resolution of the analytes in the drug mixtures, could cause problems in reduction of detection sensitivity at the trace level. This broadening can, however, be compensated through changes in the operating parameters to allow an increase in the sample stacking effect [11]. This was achieved by increasing buffer concentration (60 mM) to widen the difference in conductivity between the sample solution and the buffer. However, without a reduction in applied voltage an increase in the current results, and consequently, self-heating is problematic.

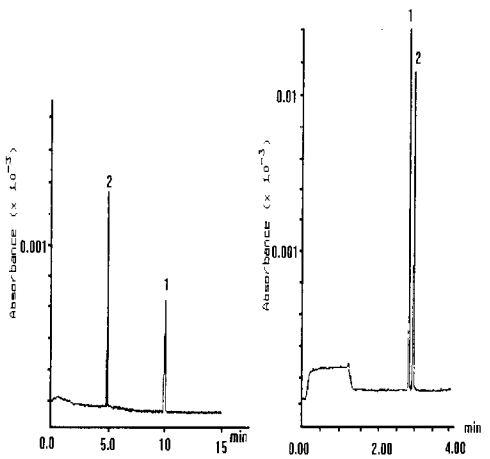

Fig. 3. The separation of the antiviral drug, acyclovir (peak 1) and its major degradation product, guanine (peak 2) on: (a) a conventional capillary (57 cm (50 cm) × 50 μm), (Beckman P/ACE 2210) and operating parameters: buffer – 20 mM sodium citrate (pH 2.5); voltage – 30 kV; wavelength – 254 nm; temperature – 25°C; (b) the polyamine coated eCAP™ (57 cm (50 cm) × 50 μm i.d.), (Beckman P/ACE 2210) and operating parameters for both were: buffer – 50 mM sodium acetate (pH 4.2); voltage – 30 kV; wavelength – 254 nm; temperature – 25°C.

However, in the case of the separation in Fig. 5b, the operating current during the run (for the 37 cm capillary) was < 100 μA which is considered to be the upper limit when caution is necessary. For this assay on the 37 cm capillary with 50 mM acetate and a voltage of 20 kV, the normal validation parameters for TMP were examined and were within the limits set of precision R.S.D. < 1% and a good linear relationship between response and concentration of 0–140% m/m (target concentration of 50 μg/ml TMP) ($r = 0.999$, $n = 8$)

It was, however, possible to reduce the overall migration time further by the two operations of: increase in voltage or the reduction again in capillary length. In the first case, an increase in voltage from 20 to 30 kV on the 37 cm capillaries gave a migration time of 1.63 min overall for the 5 components on the eCAP™, but the current was close to the 100 μA danger point (Fig. 6) and components **2** and **3** are not fully resolved in the eCAP™ capillary. However, in the case of the

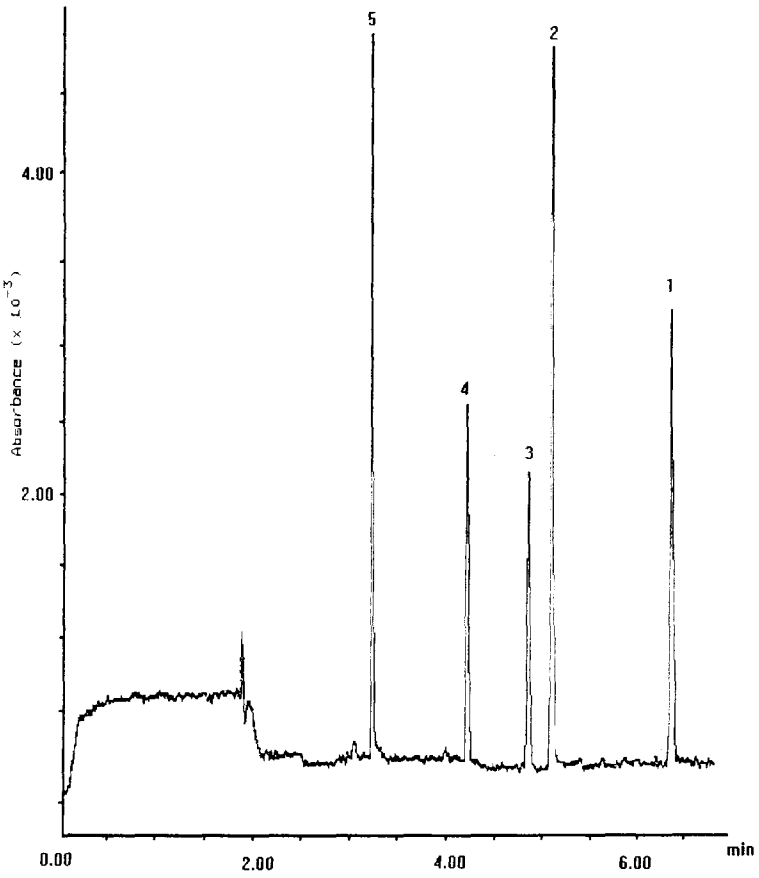

Fig. 4. The separation of TMP and the four related substances with the polyamine coated eCAP™ capillary (57 cm (50 cm) × 50 μm i.d.) where the operating parameters were: buffer – 50 mM sodium acetate (pH 4.2); voltage – 20 kV; wavelength – 254 nm; temperature – 30°C. For the key to the electrophoretic peaks see Fig. 1.

conventional capillary the resolution is unsatisfactory, even after attempted optimisation (Fig. 6a). As regards further capillary shortening on the Beckman instrument used, the capillary can be cut to a minimum length to fit the cartridge cassette of 27 cm (20 cm to detector), although the fitting of the capillary into the cartridge and its operation should be carried out with care as the capillary can be strained at this length. However, at this length it does give added advantages in migration time as shown in the comparison from the electropherograms in Fig. 7 of a basic drug test mixture of salbutamol, lamivudine, imidazole and aminobenzoic acid run at pH 8.0 on the 37 cm conventional and eCAP™ capillary against the same test mixture on 27 cm capillaries. Poor separation of the components is demonstrated on the conventional capillary in both cases. Whereas on the eCAP™ (Fig. 8) the overall migration time is down from 1.97 to 0.91 min, with acceptable resolution, although, as previously, the peak broadening is greater in the 27 cm capillary. Additionally the 37 cm capillary gives a more reproducible assay result in terms of peak area for TMP (R.S.D. < 1%) than in the case of the 27 cm (R.S.D. > 2.0%), which suggests that the broader peak width or tailing may be a quantitative measurement factor in the assay. Therefore, from this and the above results, the 37 cm capillary appears to give the most stable reproducible results and, although it has not been examined under a 6 month stringent test as with the 57 cm capillary, it

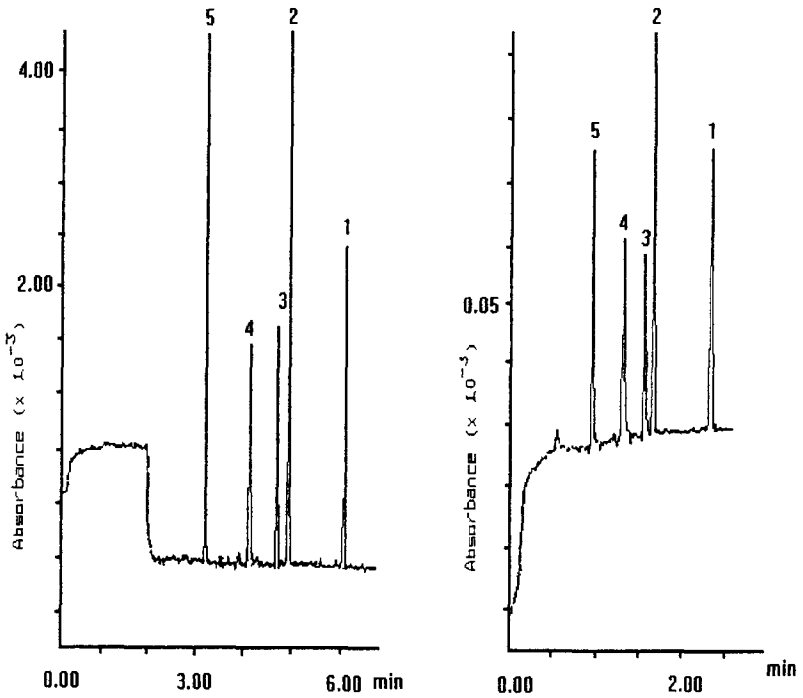

Fig. 5. Comparison between the separation of TMP and its four related substances on the polyamine coated eCAP™ on different length capillaries (a) the capillary length was 57 cm(50 cm) × 50 μm i.d. and (b) the capillary length was 37 cm (30 cm) × 50 μm i.d. For the operating parameters see Fig. 4 and for key to the electrophoretic peaks see Fig. 1.

is likely from shorter term reproducibility results that either capillary would be suitable for assay of trimethoprim in the bulk drug and in a pharmaceutical formulation.

4. Conclusion

These results are very promising for this commercially produced eCAP™ coated capillary, in terms of the performance for the basic drugs considered and for mixtures which contain mixed functionality components. Particularly impressive is the robustness and stability of the treated capillary across pH ranges and over the extended time period tested. In a number of previously described and commercial products with a treated silica surface and with those where bonding to the silica surface has been carried out, such as alkyl–silyl chains (C_1-C_{18}), there have been questions raised regarding the robustness of such columns for everyday use. However, this reasonably simple process of conditioning the surface with 1 M NaOH and then introducing a 'trade marked' polyamine as a coating with reconditioning taking place each time, appears to be an acceptable technique for basic and acidic compounds in the pharmaceutical field.

These results suggest that the coated capillary could be used for the rapid assays of bulk drug and pharmaceutical preparations for both the active and the related substances, where robustness and reproducible results over an extended time span are important. Another application which has been considered is the combination of this capillary with chiral selectors in the buffer for the resolution of chiral drugs.

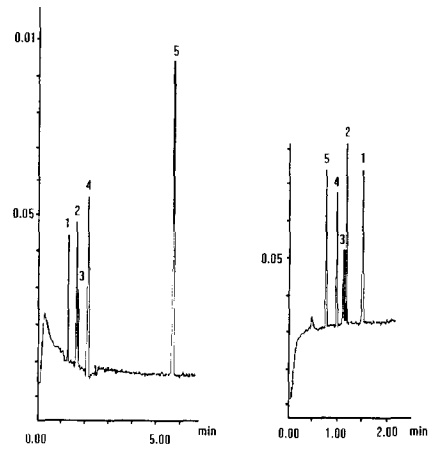

Fig. 6. Comparison between the separation on (37 cm (30 cm) × 50 μm i.d.) conventional and polyamine coated eCAP™ capillaries. For the operating parameters see Fig. 3b and for key to the electrophoretic peaks see Fig. 1.

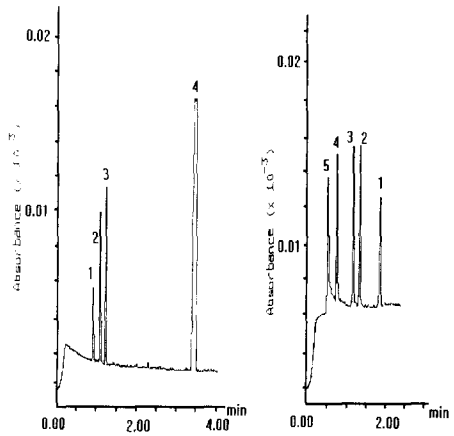

Fig. 7. Comparison of the separation of a basic drug mixture on (a) a conventional capillary (37 cm(30 cm) × 50 μm i.d.) and (b) the eCAP™ capillary (37 cm (30 cm) × 50 μm i.d.). The operating parameters for both capillaries were: buffer – 50 mM Tris (pH 8); voltage – 30 kV; wavelength – 214 nm; temperature – 30°C. The key to the electrophoretic peaks is: (1) salbutamol; (2) imidazole; (3) lamivudine; (4) aminobenzioc acid.

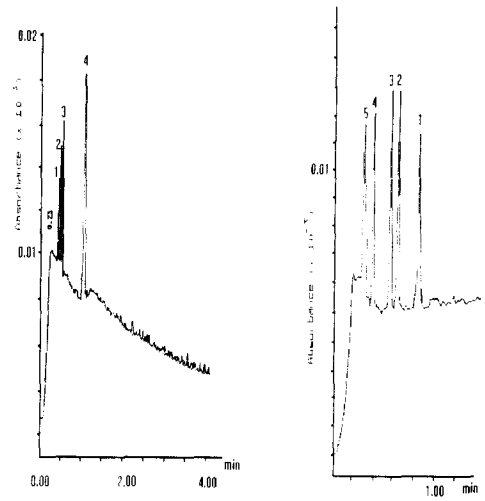

Fig. 8. Comparison of the separation of the basic drug mixture on short capillaries (a) a conventional capillary (27 cm (20 cm) × 50 μm i.d.) and (b) polyamine eCAP™ (27 cm (20 cm) × 50 μm i.d.). For operating parameters and key to electrophoretic peaks see Fig. 7.

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