Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 4/5, pp. 415–420, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00
Pergamon Press Ltd

Short Communication

Application of capillary electrophoresis as a quantitative identity test for pharmaceuticals employing automated on-column standard addition

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Keywords: *Capillary electrophoresis; pharmaceutical analysis; identity test; on-column standard addition.*

Introduction

Chemical identity confirmation is required following production of a batch of drug substance, or formulation. Typically, this is performed using a spectroscopic method and/or a combination of two separative methods. Combinations of HPLC with NMR or IR are commonly applied testing regimes.

Capillary electrophoresis (CE) is finding increasing applications within pharmaceutical analysis [1–6]. Features of CE include a high level of automation and that minimal method development is often required. These factors make CE a simple and attractive complement to an HPLC identity test.

The validation criteria of a separative method prior to its application as an identity confirmation test are as follows:

(1) Appropriate method specificity to be demonstrated, i.e. the method should be capable of resolving the test substance from other samples likely to be present, or to be confused with the compound.

(2) Concordance of test material with a designated analytical standard, i.e. the main peak in the sample chromatogram/electropherogram coresponds to the main peak in that of the standard.

(3) A quantitative indication that the test sample contains the compound of interest at the expected level.

The high resolving power [7] of CE in terms of selectivity and peak efficiency make it useful for identity confirmation purposes.

In CE, sample introduction into the capillary can be achieved by either electrokinetic [8] or hydrodynamic [9] sampling. In hydrodynamic sampling, a pressure differential across the capillary forces sample volumes, in the order of 1–20 nl, into the capillary. Sample and electrolyte ionic strengths are often selected such that a preconcentration, or 'stacking' of sample ions occurs upon application of the separation voltage [10–14]. The extent of sample stacking is related to the concentration of electrolyte [14].

This stacking phenomenon can be utilized to conduct on-column standard addition by the co-injection of both the sample and spiking solution. The sample solution is first injected, followed by an injection of the second solution plug. Application of the separation voltage causes stacking of both sample zones into a single, thoroughly mixed, tight band. This co-injection procedure has previously been employed [15] to confirm the identity of a component in the separation of a mixture of drugs. However, the quantitative aspects of co-injection have not been demonstrated in terms of the effect on resolution, separation efficiency and quantitative peak area measurements.

It was decided to investigate whether CE,

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Table 1
Separation method used for analysis

Step I	Rinse cycle 1	0.5 M NaOH (2 min)
Step II	Rinse cycle 2	20 mM Na citrate pH 2.5 (2 min)
Step III	Set detector	0.02 AUFS, 5 Hz data collection
Step IV	Sampling	5 s (from vial in position X)
Step V	Sampling	2 s (from sample vial)
Step VI	Operating voltage	+30 kV
	Run time	10 min
	Wavelength	200 nm
	Capillary	75 μm × 57 cm (50 cm to detector)

employing standard addition by co-injection, would be a suitable alternative and/or complement to HPLC as a quantitative identity test.

Experimental

Inorganic chemicals were purchased from Aldrich Ltd (Poole, Dorset, UK). Water was obtained from a Millipore Q system (Watford, Herts, UK). Capillary electrophoresis was performed on a P/ACE 2000 CE instrument (Beckman, Palo Alto, CA, USA) which was connected to a Hewlett–Packard (Bracknell, Berks, UK) data collection system. The separation method used for analysis is given in Table 1.

Results and Discussion

On-column standard addition by co-injection

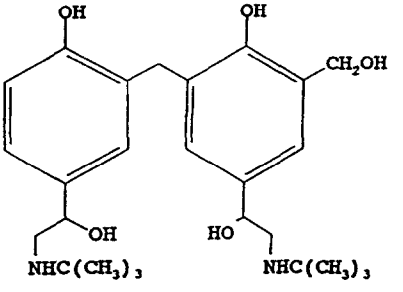
To automatically spike a sample (on-column) with an impurity a vial containing a solution of the impurity is placed on the autosampler at position X (see Table 1).

Effect of co-injection on system performance

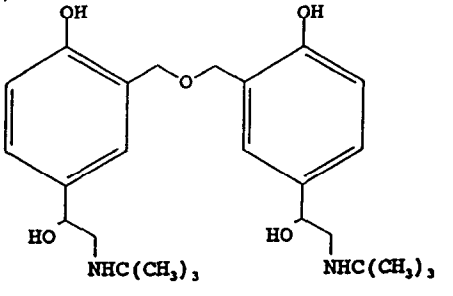
Previously the cross-correlation between CE, TLC and HPLC of levels of two selected dimeric impurities ('bis ether' and 'side-by-side dimer') present in the pharmaceutical salbutamol (Fig. 1 gives the structures of the compounds involved) have been reported [16]. This separation was selected to demonstrate the effect of co-injection.

Figure 2(a) shows the CE separation of a typical salbutamol drug substance batch (1 mg ml^{-1} in water) containing both the bis ether and side-by-side dimer impurities present at ~0.2% w/w of the salbutamol loading. This separation was achieved using the conditions given in Table 1 with Step IV set to 0 s. Figure 2(b) shows the separation of the same sample spiked on-column with 0.5% w/w of the bis ether impurity. This spiking was achieved using

(I)



(II)



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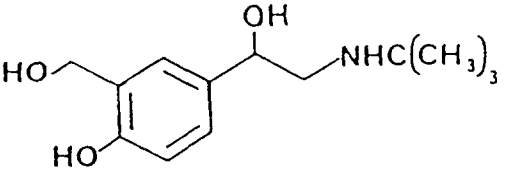

Figure 1
I, 'Dimer' impurity of salbutamol; II, 'bis ether' impurity of salbutamol; and III, salbutamol.

the method as detailed in Table 1. It can be seen that there is no loss in resolution or separation efficiency with the co-injection method.

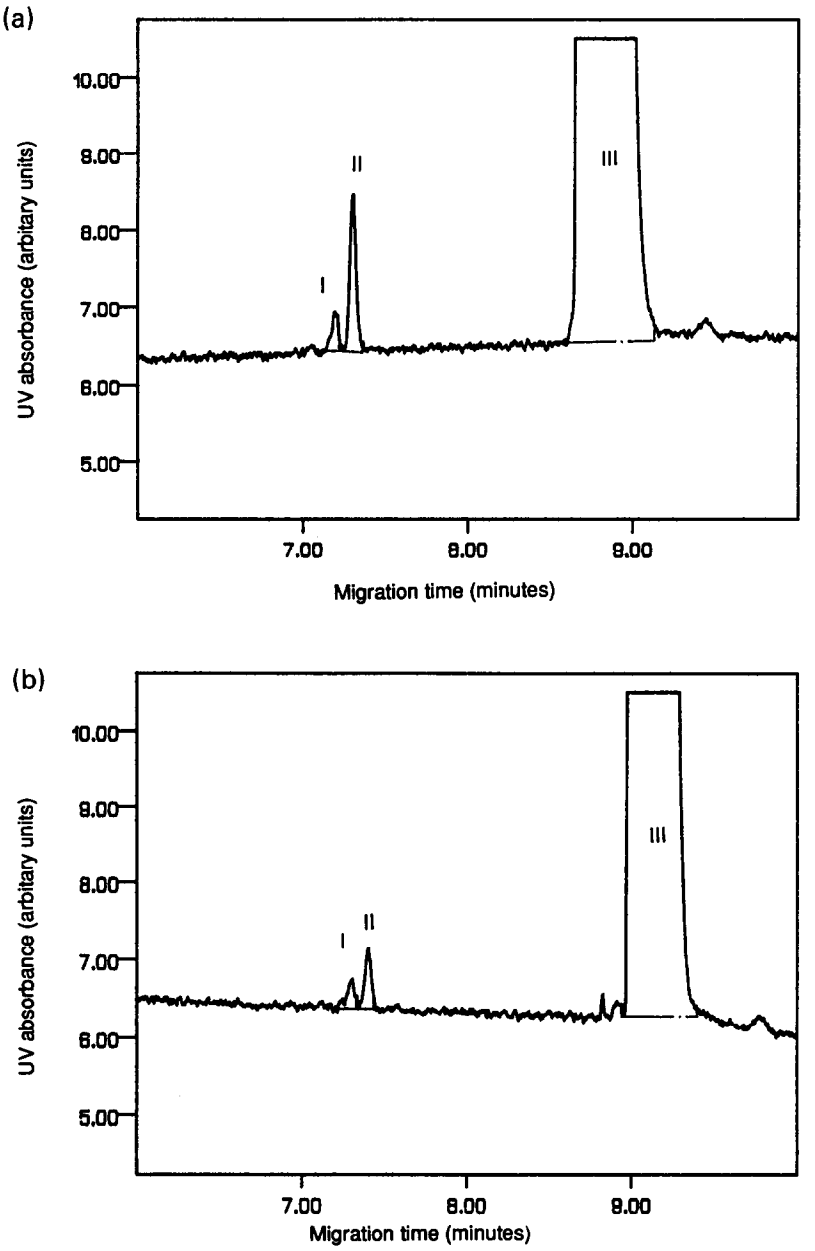

Figure 2
(a) CE separation of a 1 mg ml^{-1} salbutamol solution. (b) CE separation of a 1 mg ml^{-1} salbutamol solution spiked with 0.5% w/w bis ether by co-injection. Separation conditions as given in Table 1, chemical structures given in Fig. 1.

Linearity of co-injection

By modifying the injection times given in Table 1 it is possible to 'automatically' cover a range of spiking levels by preparing only a single impurity standard and a sample solution. For example, a 1 mg ml^{-1} salbutamol sample was spiked on-column with a bis ether solution in the range 0.1–0.5% w/w of the salbutamol loading. To achieve this it was necessary to prepare a 0.1% w/w bis ether solution. The salbutamol solution injection time was maintained at 2 s whilst the co-injection time of the impurity standard was varied between 2 and 10 s (equivalent to spiking levels of 0.1–0.5% w/w). Figure 3 shows the linear increase in bis ether peak area with co-injection sampling time. Figure 3 also shows that the other dimeric impurity (side-by-side) peak area remains constant throughout the analyses.

Quantitative performance of co-injection

To assess the validity of this approach a 1 mg

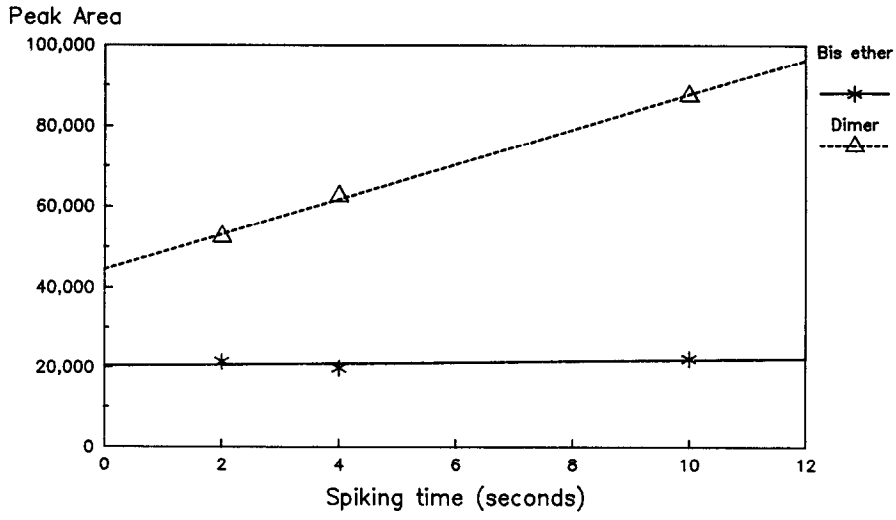

Figure 3
Plot of bis ether and side-by-side peak areas versus sampling time.

Table 2
Correlation between peak areas for samples spiked manually or by co-injection

	Bis ether peak area	
Sample	Manually	Co-injection
Salbutamol spiked with 0.10% w/w	3244	3324
Salbutamol spiked with 0.95% w/w	9885	9768

ml^{-1} salbutamol sample was manually and 'automatically' spiked with a solution of bis ether at levels of 0.10 and 0.95% w/w. The correlation between the peak areas is shown in Table 2. The variation between results is within the experimental error observed when determining low impurity levels.

Illustrative application of CE as an identity confirmation test

Quinolone type compounds are being extensively explored for use as antibiotics [17]. A quinolone antibiotic has been investigated within Glaxo. A CE-based method has been developed for identity confirmation to supplement IR data.

The following testing regime is employed to confirm identity by CE:

(1) A 0.1 mg ml^{-1} solution of authentic drug standard (dissolved in water) is electrophoresed under the conditions given in Table 1 with Step IV set to 0 s.

(2) A 0.1 mg ml^{-1} solution of the test material (dissolved in water) is analysed under the conditions given in Table 1 with Step IV set to 0 s.

(3) A co-injection of the test solution (5 s) and standard solution (5 s) is performed under the conditions given in Table 1.

By using this regime, it is possible to verify concordance of the sample with the analytical standard. Quantitation is possible by noting the peak areas of the individual injections of the solutions containing the test and authentic material. Comparisons with the sum of these two areas with the peak area obtained from the analysis of the mixture (spiked) solution should indicate a quantitative increase in peak area.

Figure 4(a) shows the analysis of co-injection of both sample and standard solutions. The analysis of the single injections of the sample and standard solutions are shown in Fig. 4(b) and (c), respectively. The area of the spiked sample was equivalent to the sum of the individual peak areas for the standard and test solution. The analytical sequence was performed using the associated PC controller and was run unattended.

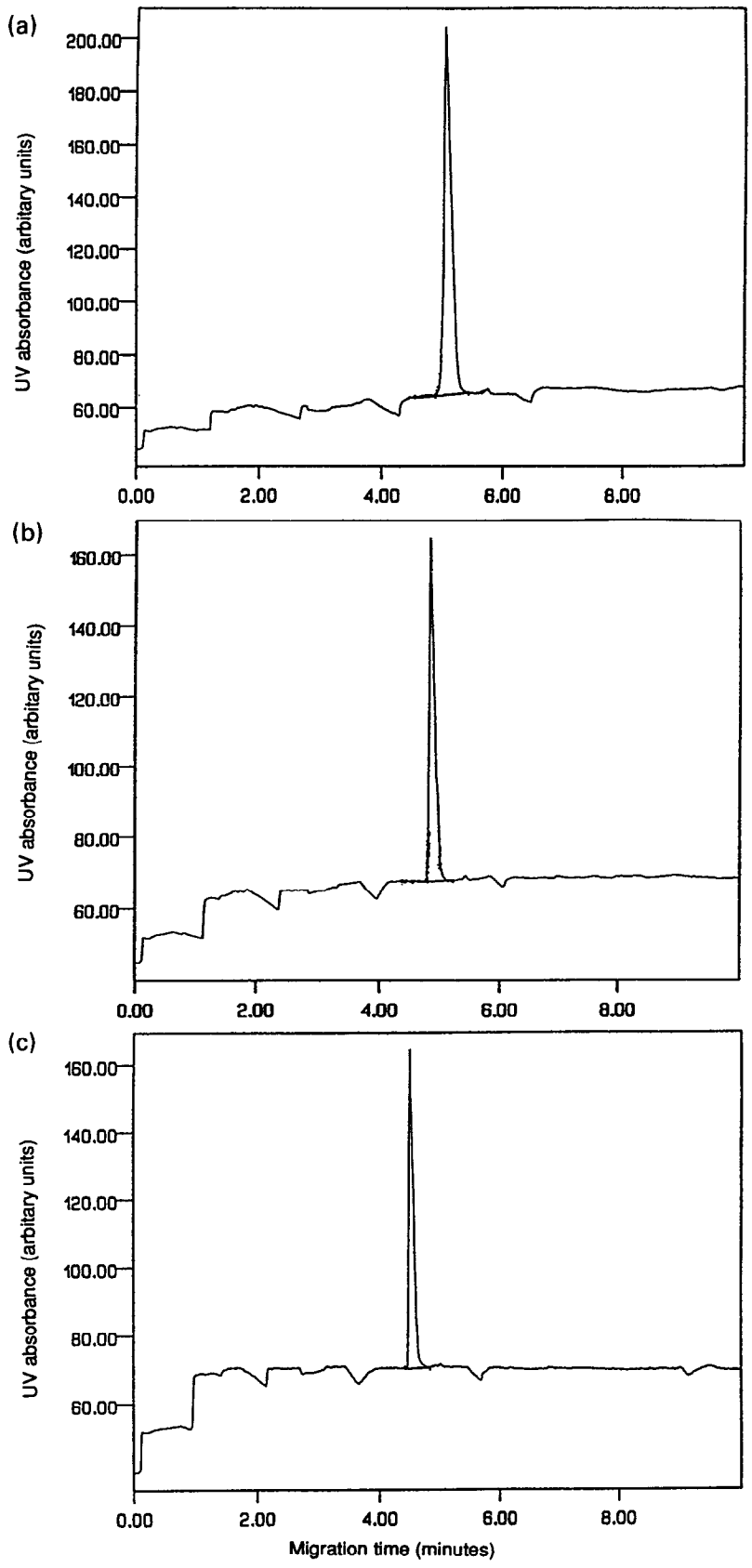

Figure 4
Electropherogram of (a) 'on-capillary' spiked sample, (b) standard solution, and (c) sample solution. Separation conditions as given in Table 1, except that detection wavelength was 254 nm.

Conclusions

CE can offer a simple complement to HPLC-based identity tests for both drug substance and formulations. Fully automated CE systems allow an identity test to be preprogrammed and to run unattended.

Use of a co-injection sampling regime has been found to have no effect on either peak efficiency or resolution. Variable co-injection was shown to give a linear increase of peak area with sampling time.

Co-injection is shown to be of benefit to quantify and identity components of interest in a sample as this procedure avoids the need to manually prepare a range of samples spiked with appropriate levels of the component of interest. This may be of particular importance when dealing with valuable samples as contamination of the test sample is avoided.

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[Received for review 20 August 1992; revised manuscript received 7 October 1992]