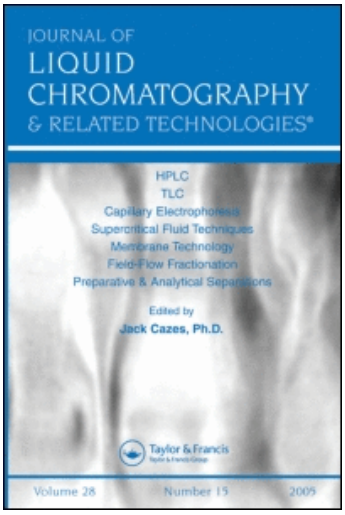


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitative Pharmaceutical Analysis by Capillary Electrophoresis

K. D. Altria[a]; S. D. Filbey[a]
[a] Pharmaceutical Analysis, Glaxo Group Research, Herts, United Kingdom

To cite this Article Altria, K. D. and Filbey, S. D.(1993) 'Quantitative Pharmaceutical Analysis by Capillary Electrophoresis', Journal of Liquid Chromatography & Related Technologies, 16: 11, 2281 — 2292
To link to this Article: DOI: 10.1080/10826079308020985
URL: http://dx.doi.org/10.1080/10826079308020985

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(11), 2281-2292 (1993)

QUANTITATIVE PHARMACEUTICAL ANALYSIS BY CAPILLARY ELECTROPHORESIS

K. D. ALTRIA AND S. D. FILBEY
Pharmaceutical Analysis
Glaxo Group Research
Park Road
Ware, Herts, United Kingdom SG12 ODP

ABSTRACT

CE has been employed for the quantitative determination of sumatriptan levels in subcutaneous injection solutions. Results generated by both CE and HPLC for four batches of sumatriptan Injection solutions compared well. The CE method gave good performance in terms of selectivity, precision, linearity and repeatability of both injection and analysis. This paper provides an example of the employment of quantitative CE within a working industrial environment.

INTRODUCTION

Capillary Electrophoresis (CE) has been investigated (1-12) for a number of applications within the area of pharmaceutical analysis. However, there has been little emphasis on demonstrating that these methods are capable of routinely determining the drug content of formulated pharmaceuticals in working analytical environments.

This paper describes the preliminary validation experiments, and application of a CE method, for the determination of sumatriptan contents in subcutaneous injection solutions. The results obtained by this CE method are compared with those generated by a HPLC method.

EXPERIMENTAL

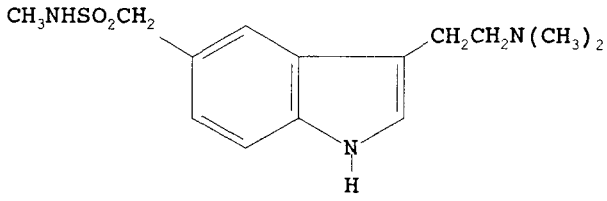
Chemicals were obtained from Aldrich Ltd.(Poole, Dorset, UK), and water was obtained from a Millipore Q system (Watford, Herts., UK). The quantitative work was performed on a Waters Quantum 4000 CE instrument (Watford, Herts., UK) which was connected to a Hewlett Packard data collection system (Bracknell, Berks., UK). High speed analysis and comparative separations were achieved on a P/ACE 2000 CE instrument (Beckman, Palo Alto, CA, US). The fused silica capillaries used in this study were purchased from both Waters and Beckman.

In this work an internal standard was employed since this has been shown to improve the repeatability of injection in CE (13). A precursor (14) to ranitidine (15) was selected (chemical structures given in Figure 1) as this was known to migrate before any sumatriptan related compounds. Sample and standard solutions were prepared to give a final aqueous concentration of 0.5mg/ml of both internal standard and sumatriptan.

RESULTS AND DISCUSSION

Sumatriptan is marketed for the treatment of migraine (16). Two formulations are marketed, a subcutaneous injection solution and tablets. It was decided to investigate the potential of CE to quantify levels of sumatriptan in injection solutions. The samples selected for this purpose contained sumatriptan formulated at 12mg/ml in isotonic saline solution.

Sumatriptan



Internal standard

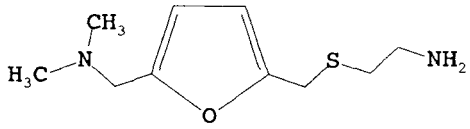


FIGURE 1

Chemical structures of sumatriptan and internal standard

Currently HPLC methods are employed for the determination of both sumatriptan and related impurities content in subcutaneous sumatriptan injection solutions.

Method development

Practical guidelines to the method development options for CE of pharmaceuticals have recently been published by McLaughlin et al (17). For this particular separation a low pH (pH 2.3) was selected to ensure protonation of both the analyte and related impurities.

Figure 1 shows the CE separation of a synthetic test mixture of sumatriptan, a dimeric related impurity, and the internal standard. This method therefore offers the possibility of determining both dimeric impurity

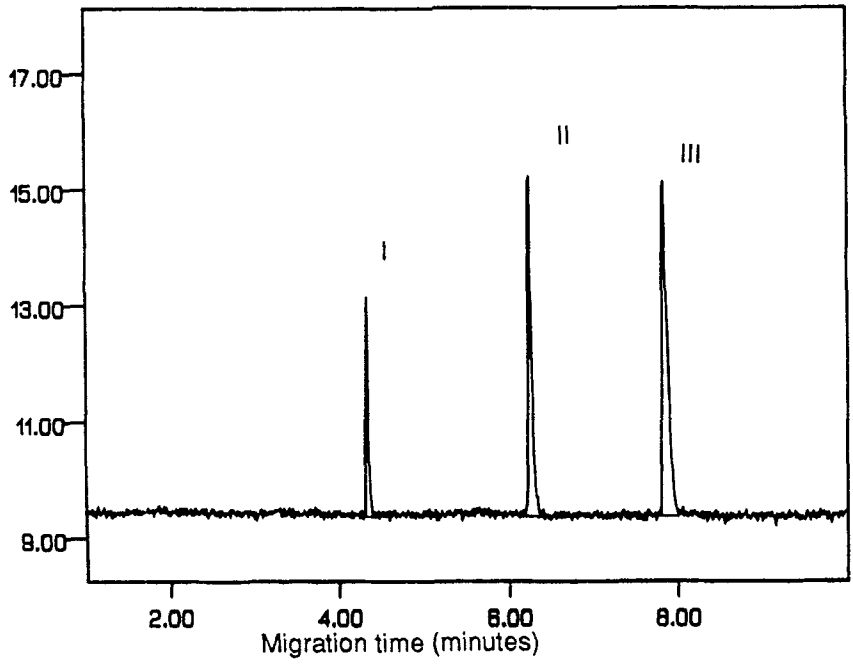


FIGURE 2

CE separation of internal standard (I), sumatriptan related dimeric impurity (II) and sumatriptan (III). Separation conditions : 20.0 seconds hydrodynamic sampling, +20kV, 214 nm, 75μm x 60cm fused silica capillary, sodium dihydrogen orthophosphate (25mM, pH 2.3 with conc. phosphoric acid)

levels as well as sumatriptan content. The cross-correlation between CE and HPLC for the determination of dimeric impurities in salbutamol drug substance material has previously been reported (18) .

Precision of injection

There have been several reports (19-22) concerning the reproducibility of peak areas on automated CE instruments. Equipment manufacturers typically quote that RSD's of less than 2% can be routinely obtained for

peak areas. By employing an internal standard, variability can be reduced still further with typical RDS's of below 1% being obtained (13).

Figure 3 and Figure 4 shows replicate electropherograms for calibration solutions and sample solutions respectively. These separations indicate the consistent impurity profiles obtained throughout these studies. Both a calibration and sample solution were injected 5 times and acceptable precision for peak area and peak area ratios were obtained (Table 1).

Migration time variation using the CE method , measured in terms of migration time and relative migration time,was typically less than 1% RSD.

Sensitivity

The performance of the method in terms of sensitivity was measured and a limit of detection of 0.1%w/w of the sumatriptan loading (0.5mg/ml) was obtained (signal to noise ratio greater than 3). A similar detection limit of 0.1%area/area for salicylamide impurities by CE has been reported (1). This limit of detection is equivalent to 500ppb sumatriptan in solution. This figure is in agreement with those reported previously for salbutamol a pharmaceutical having similar UV extinction characteristics (12,18).

Linearity

The linearity of detector response between 0 and 150% of the sample concentration (0.5mg/ml sumatriptan) was established. The data showed good linearity for both sumatriptan peak area and peak area ratio (correlation coefficients 0.9992 and 0.9993 respectively).

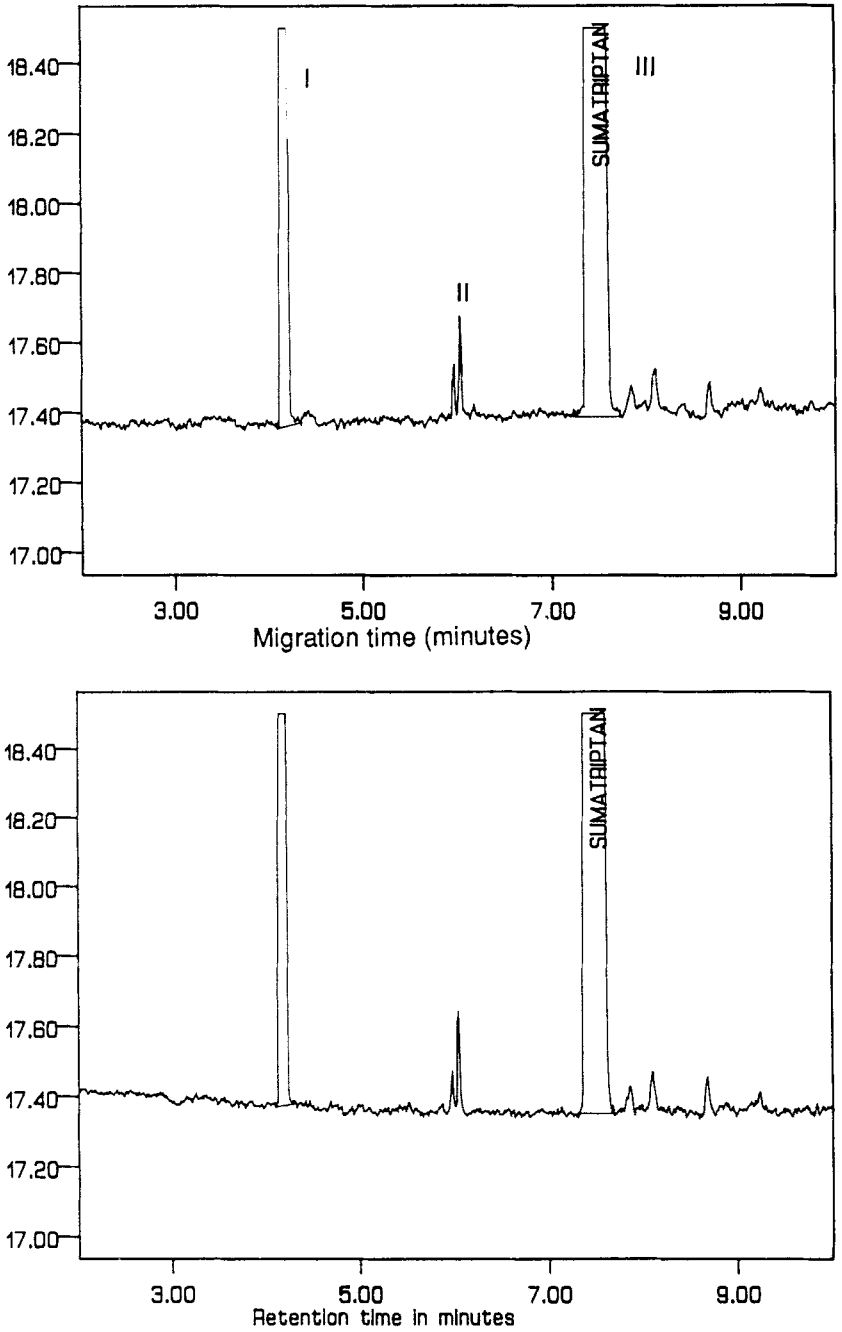


FIGURE 3a and 3b

Duplicate CE separations of a calibration solution. Separation conditions : as Figure 2

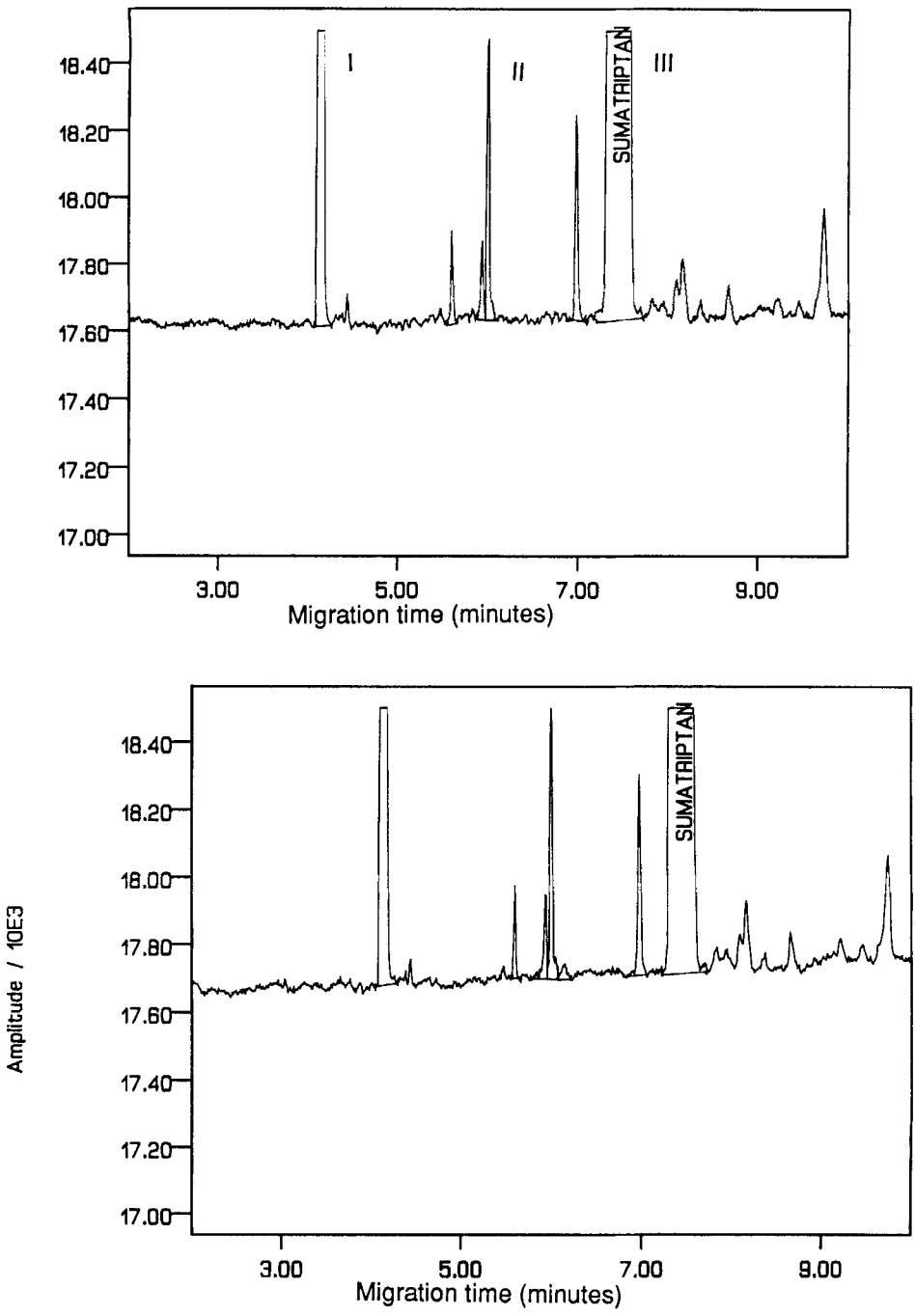


FIGURE 4

Duplicate CE separations of a sample solution Separation conditions : as Figure 2

TABLE 1

Precision of injection peak area
(number of injections = 5)

	Standard solution RSD (%)	Sample solution RSD (%)
Sumatriptan	0.7	0.7
IS	0.5	0.1
Peak area ratio	0.5	0.8

Table 2
Repeatability of results day-to-day

Analysis set 1

	Sumatriptan content (mg/ml)			
Sample	CE results			HPLC
Batch 1	Day 1	Day 2	Mean	
Condition 1	11.9	11.9	11.9	11.8
Condition 2	11.8	11.6	11.7	11.7
Condition 3	11.7	11.7	11.7	11.7

Analysis set 2

	Sumatriptan content (mg/ml)			
Sample	CE results			HPLC
Batch 2	Day 1	Day 2	Mean	
Condition 1	11.9	11.6	11.8	11.6
Condition 2	11.7	11.6	11.7	11.7

Repeatability of analysis

The day-to-day variability of analysis for sumatriptan content was established by conducting two separate sets of analyses on two separate occasions. Similar results for each sample set were obtained on each occasion and these were in agreement with those achieved by HPLC (Table 2). Each analysis set comprised testing of on-going stability batches which had been stored at various conditions of heat and humidity.

Table 3

Sumatriptan content by CE and HPLC

Sample	Sumatriptan content (mg/ml) CE	HPLC
Batch 2		
Condition 1 (aliquot 1)	11.5	11.6
Condition 1 (aliquot 2)	11.6	11.6
Condition 2 (aliquot 1)	11.6	11.7
Condition 2 (aliquot 2)	11.6	11.7
Batch 3		
Condition 1 (aliquot 1)	11.7	11.8
Condition 1 (aliquot 2)	11.8	11.8
Condition 2 (aliquot 1)	11.6	11.7
Condition 2 (aliquot 2)	11.6	11.7
Batch 4		
Condition 1 (aliquot 1)	11.7	11.8
Condition 1 (aliquot 2)	11.8	11.8
Condition 2 (aliquot 1)	11.7	11.7
Condition 2 (aliquot 2)	11.6	11.7

Repeatability of separation

In order to assess the ruggedness of the method the separation was performed on an alternative CE instrument using a capillary from a different supplier. The separation achieved showed an identical migration order to that achieved on the earlier instrument and capillary.

Cross-correlation between sumatriptan content results by CE and HPLC

Currently HPLC is employed for the determination of sumatriptan contents (23). Sumatriptan content was determined by CE for three stability batches of Sumatriptan (12mg/ml) Injections using external standardisation. Table 3 shows the comparison of the results obtained by CE and HPLC. Two aliquots were taken from each sample, and each

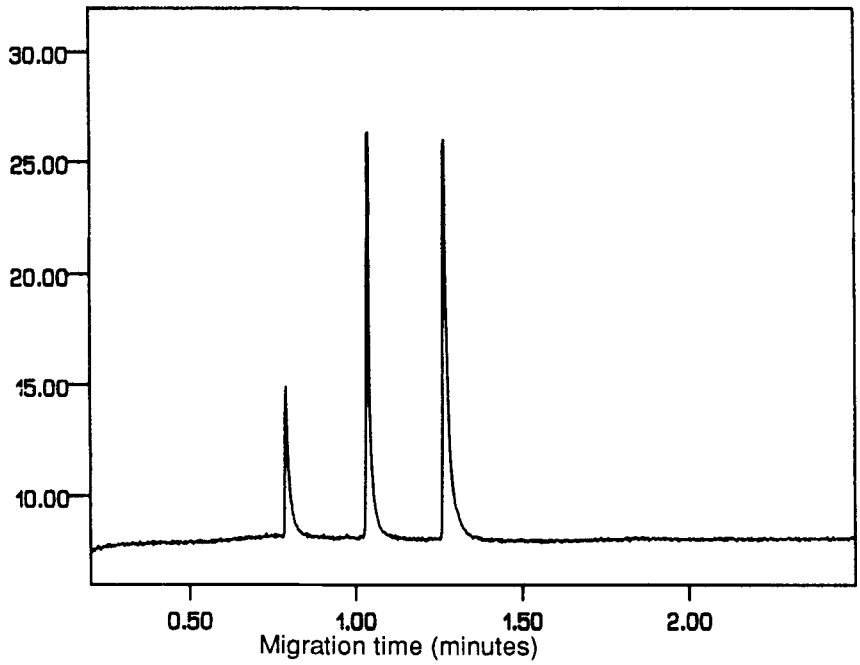


FIGURE 5

HSCE separation of test mixture Separation conditions : as Figure 2 except 25cm x 50μm capillary and 5 seconds sampling time

aliquot was analysed in duplicate. The results reported below are the mean of the two injections of each aliquot.

Optimisation of analysis time

The use of high field strength applied across short capillaries can dramatically reduce analysis times. Figure 5 shows the separation of the 3 component test mix using identical conditions except applying +30kV across a 25cm X 50μm capillary. It should be noted that the separation profile is identical to that achieved in Figure 2 although the separation

time is greatly reduced. High Speed Capillary Electrophoresis (HSCE) has the potential to dramatically increase sample throughput.

CONCLUSIONS

A CE method has been employed for the quantitative determination of sumatriptan content in subcutaneous injection solutions. Method validation has been performed which has included measurements of the injection precision of peak areas, peak area ratios and migration time. Other validation aspects examined included linearity, sensitivity, and repeatability of both analysis and separation.

The sumatriptan content results for 4 stability batches at different storage conditions showed excellent agreement between CE and HPLC. This exercise gave useful information on the validity of both methods, demonstrating the complimentary nature of CE and HPLC.

REFERENCES

1. Swartz M.E., *J.Liq.Chromatogr.*, 14: 923-938 (1991)

2. Roach M.C., Gozel P. and Zare R.N., *J.Chromatogr.*, 426: 129-140 (1988)

3. Hoyt A.M. and Speniak M.J., *Anal.Letters*, 861-873 (1989)

4. Altria K.D. and Simpson C.F., *J.Biomed.Pharm.Anal.*, 6: 801-807 (1988)

5. Altria K.D. and Smith N.W., *J.Chromatogr.*, 538: 506-509 (1991)

6. Fujiwara S. and Honda S., *Anal.Chem.*, 59: 2773-2776 (1987)

7. Swaile D.F., Burton D.E., Balchunas A.T. and Sepaniak M.J., *J.Chrom.Sci.*, 26: 406-409 (1988)

8. Nishi H., Tsumagari N., Kakimoto T. and Terabe S., *J.Chromatogr.*, 477: 259-270 (1989)

9. Nagawaka T., Oda Y. and Shibukawa A., *Chem.Pharm.Bull.*, 37: 707-711 (1989)

10.Nishi H. Fukuyama T. Matsuo M. and Terabe S., *J.Pharm.Sci*, 79: 519-523 (1990)

11. Nishi H. and Terabe S., *Electrophoresis*, 11: 691-701 (1990)

12. Ackermans M.T., Beckers J.L., Everaerts F.M. and Seelen I.G.J.A., *J.Chromatogr.*, 590: 341-353 (1992)

13. Dose E.V. and Guiochon G.A., *Anal.Chem.*, 63: 1154-1158 (1991)

14. Evans M.B., Haywood P.A., Johnson D., Martin-Smith M., Munro G. and Wahlich J.C., *J.Pharm.Biomed.Analysis*, 7: 1 (1989)

15. Dawson J., Richards D.A., Stable R., Dixon G.T. and Cockel R., *J.Clin.Hosp.Pharm.*, 8: 1 (1983)

16. Doenicke A., Brand J. and Perrin V.L., *Lancet*, 8598: 1309-1311 (1988)

17. McLaughlin G.M., Nolan J.A., Lindahl J.L., Morrison J.A. and Bronzert T.J., *J.Liquid Chromatogr.*, 15: 961-1021 (1992)

18. Altria K.D., *J.Chromatogr.*, accepted in press

19. Rahn P.C., *Am.Biotechnol.Lab.*, 22-29 (1990)

20. Schwartz H.E., Melera M. and Brownlee R.G., *J.Chromatogr.*, 480: 129-139 (1989)

21. Rose D.J. and Jorgenson J.W., *Anal.Chem.*, 60: 642-648 (1988)

22. Honda S., Iwase S. and Fujiwara S., *J.Chromatogr.*, 404: 313-320 (1987)

23. Oxford J. and Lant M.S., *J.Chromatogr.*, 496: 137-146 (1989)

Received: September 22, 1992
Accepted: November 5, 1992