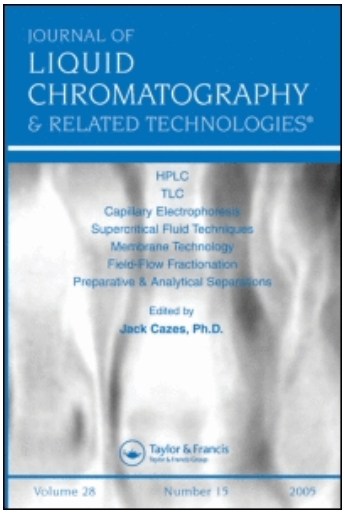

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K. D. Altria[a]; E. Creasey[a]; J. S. Howells[a]
[a] Pharmaceutical Development (Europe) GlaxoWellcome Research and Development Park Road Ware, Herts, U.K.

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ROUTINE CAPILLARY ELECTROPHORESIS TRACE LEVEL DETERMINATIONS OF PHARMACEUTICAL AND DETERGENT RESIDUES ON PHARMACEUTICAL MANUFACTURING EQUIPMENT

K. D. Altria, E. Creasey, J. S. Howells

Pharmaceutical Development (Europe)
GlaxoWellcome Research and Development
Park Road
Ware, Herts., SG12 ODP, U.K.

ABSTRACT

Capillary electrophoresis methods are reported for the determination of trace levels of substances possibly present as contaminants on equipment used in pharmaceutical manufacturing. A previously reported method for the determination of a range of basic drugs was optimised through use of internal standards and a shorter length capillary. This method was used to establish an optimised extraction procedure through analysis of solutions containing a range of drugs with different solubilities. A CE method for the trace level analysis of a range of different acidic drugs was shown to be capable of achieving acceptable precision and sensitivity. Levels of detergent solution residue were monitored by quantifying potassium levels using indirect UV detection. These methods are in routine use within our laboratories.

INTRODUCTION

The use of capillary electrophoresis to monitor trace level of contaminants is becoming increasingly established in many application areas.[1-12] The sensitivity requirements for these applications are often at the low ppm (mg/L) or ppb (μg/L) levels. Several approaches to obtaining these detection levels in CE have been adopted including the use of solid phase extraction[1] and combinations of low wavelength UV detection and wide bore capillaries.[2]

The number of samples involved in trace level determinations is often high and therefore a short analysis time is a necessity. Short capillary lengths and/or high voltages have been widely used to reduce analysis times in CE. Reported trace level determinations have included a low pH CE method for aromatic amines in water samples,[3] or high pH electrolytes for determination of both acidic herbicides,[4] surfactants[5] and aromatic sulfonic acids.[6] Micellar electrokinetic capillary chromatography (MECC) methods have been used to separate complex mixtures of neutral and charged components such as explosive residues,[7] herbicide residues[8] and phenols/polynuclear aromatics.[9] Trace level determinations of non-chromophoric species have been achieved through use of indirect UV detection.[10,11]

Trace level contamination is an important issue in the pharmaceutical industry, especially with the increasing potency of many drugs which may be therapeutically active at the μg level. The main emphasis of trace level determination in pharmaceutical analysis is to demonstrate that manufacturing equipment is sufficiently cleaned prior to the next use of the equipment for manufacture. This testing, known as cleaning qualification (CQ) analysis, is mainly necessary to prevent the possibility of any cross-contamination of products. Following cleaning of the manufacturing equipment the cleanliness of the equipment is assessed analytically. This assessment generally involves wiping areas of the cleaned equipment with pieces of material such as cotton wool, nylon filters, or filter paper. The piece of material, known as a swab, is moistened with an appropriate solvent. The swab is then extracted with a known volume of a dissolving solvent. The resulting solution is then analysed for drug content.

Removal of water-soluble drug residues from equipment used in manufacture is generally sufficiently effective using water as the solvent. However, use of detergent solutions is often required to remove residues of water-insoluble drug substances. When detergent solutions are employed it is necessary to remove all detergent residues to a sufficiently low level and further analytical testing is required to demonstrate this. Detergent solutions typically contain a range of components which may include organic solvents, surfactants,

and chelating agents. Detergent solutions may also be highly caustic and contain high levels of alkalis such as NaOH or KOH. Detection of one of the detergent solution components is selected to allow monitoring of the detergent solution residues.

The sensitivity required to monitor drug and detergent solution residues on pharmaceutical manufacturing equipment is in the low mg/L to mid ng/L range. These sensitivity levels can be obtained using optimised capillary electrophoresis methods and the use of CE to monitor residues of basic drugs[2] and surfactant residues[12] has been shown. A range of basic drugs was previously quantified on a 100 micron capillary using a pH 2.5 phosphate buffer with detection at 200nm.[2] Residues of a particular detergent solution which contained sodium dodecylbenzenesulphonate were monitored[12] using a high pH buffer with detection at 200nm.

The CE method for determination of a wide range of basic drugs residues offered significant advantages over the use of a number of different HPLC methods in terms of reduced costs and improved working practices. However, the method suffered from relatively long analysis times for some larger basic compounds and gave poorer injection precision than equivalent HPLC methods. In addition an alternative CE method was also required for determination of acidic drug residues.

The CQ method previously reported for surfactant residues monitored levels of sodium dodecylbenzenesulphonate (SDBS) which is present in a number of detergent solutions used. However, many caustic detergent solutions do not contain SDBS but do contain alkalis such as NaOH or KOH. The cleanliness of the equipment can therefore be monitored by determining the trace metal ion levels on manufacturing equipment by CE using indirect UV detection.

The ability to monitor simultaneously several different drugs allowed a thorough investigation of sampling techniques. A mixture of a range of drugs with different water solubilities was used to assess the impact of both sample extraction procedures and the type of material used as the swab. This multi-drug approach maximised the quality of information generated within the minimum number of experiments.

The focus of this paper is the further optimisation of previously reported CQ methods and also the development and validation of alternative methods to determine acidic drug residues and residues of a detergent solution specifically containing KOH.

The precision of the low pH phosphate buffer method for determination of basic drug residues was improved significantly by the use of appropriate internal standards. The use of an internal standard has been shown to be the most effective means of improving injection precision.[13] Analysis times were reduced by use of a shorter capillary length with appropriate adjustment of the field strength.

Borate buffer is widely used for the determination of acidic drugs.[14] The buffer has a natural pH of 9.4 and also allows use of low wavelengths such as 190nm.[14] A borate buffer, in combination with low UV wavelength detection and a 100 micron capillary, were successfully evaluated for determination of residues of a range of acidic drugs. Internal standards were used to give the required precision.

The use of indirect detection is common in CE for the determination of metal ions.[15] A standard CE method was modified to determine trace levels of potassium ions which were indicative of residues of the detergent solution. Magnesium was used as an internal standard in this application.

Performance data from these methods is reported for a number of routine examples. The methods were validated and are now in routine use in a number of our laboratories world-wide as cost efficient alternatives/replacements for HPLC or other test methods.

EXPERIMENTAL

Validation experiments were performed on a number of Beckman (Fullerton CA) CE instruments. A Hewlett Packard (Bracknell, Berks.) LAS 1000 data collection system was employed for integration and data handling. Inorganic chemicals were obtained from BDH (Poole, Dorset). Water was obtained from a Millipore Q system (Watford, Herts).

Capillaries were purchased from Composite Metal Services (Hallow, Worcs.) and were fitted into CE capillary cartridges with detection apertures of 100 x 800μm. Capillary detection windows were generated using an electrical filament device purchased from Capital HPLC, Broxburn, Edinburgh. Short (0.2 minute) rinse times are necessary when using short, wider bore capillaries as the back-pressure of the capillary is small and only a few seconds are required to flush the capillary contents with buffer. Extended flushes can rapidly lead to emptying of the vials containing the rinse electrolyte.

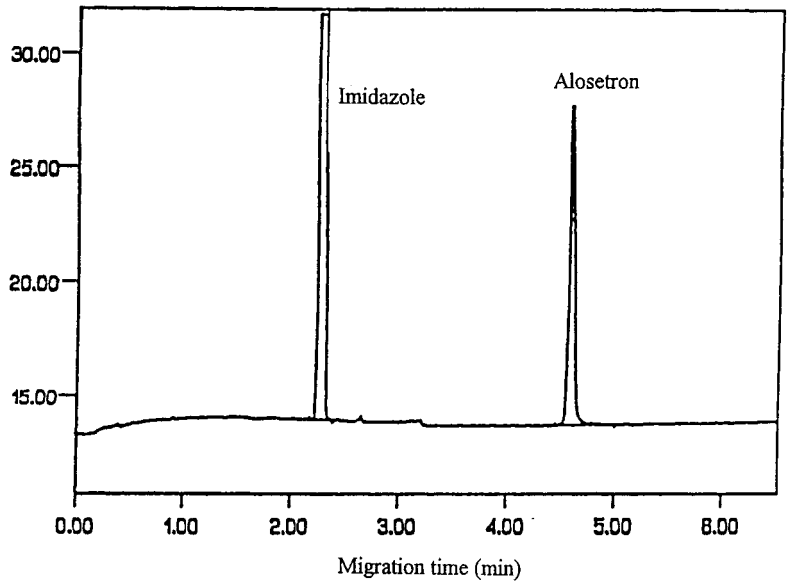

Figure 1. Separation of an Alosetron standard. Separation conditions: 27cm x 100μm capillary (20cm to detector), 200nm, 7kV, 25mM NaH_2PO_4 pH 2.3, 30C, sample 0.1μg/mL Alosetron in 0.1μg/mL Imidazole.

RESULTS AND DISCUSSION

Basic Drugs Analysis

The previously reported method was optimised and modified to improve injection repeatability and to reduce analysis times. Imidazole and aminobenzoate were generally used as appropriate internal standards. These internal standards are added at 100μg/L levels into the solvent used to prepare both the calibration solutions and to extract the drug from the swab sample.

Analytical performance

The sensitivity of the method was indicated by the basic drug, Alosetron, where limits of detection were 0.02μg/mL detected at 200nm. This is equivalent to 0.2μg/swab as an extraction volume of 10mL was used. The limit of quantitation was determined as 0.07μg/mL. Imidazole was used as the internal standard for Alosetron. Figure 1 shows separation of a calibration

Table 1

Injection Precision for a Range of Basic Drugs at Two Different Concentrations*

50μg Lamivudine	Ondansetron	Salbutamol	GW1**
1.15%	1.76 %	0.97%	1.34%
250μg Sumatriptan	**Salbutamol**	**Ranitidine**	**Lamivudine**
0.70%	0.49%	1.57%	0.11%

* (n = 10)

**GW1 is a basic drug currently under development within GlaxoWellcome.

solution. Precision values considerably below the required 5% RSD were routinely obtained for applications when using internal standards. Table 1 shows precision data obtained for calibration solutions of a number of basic drugs at two concentration levels, all containing imidazole as an internal standard. Acceptable injection precision was obtained for all components and was improved at higher drug concentrations.

Linearity was demonstrated by analysing, in duplicate, five Alosetron standard solutions covering the range 0.1-10μg/mL. A correlation coefficient of 0.99997 was obtained with an intercept value of -0.8% of the 10μg/mL standard.

Similar performance levels have been routinely obtained for a wide range of basic drugs. The method has been successfully employed in a number of laboratories using a range of instrument types and locally sourced reagents and capillaries.

Water insoluble drugs are extracted and prepared using either acidified water, diluted electrolyte (typically 1 to 10 dilution with water) or aqueous-organic solvents. These diluents are listed in terms of preference as the choice of sample diluent has a pronounced effect on the quality of separation obtained.

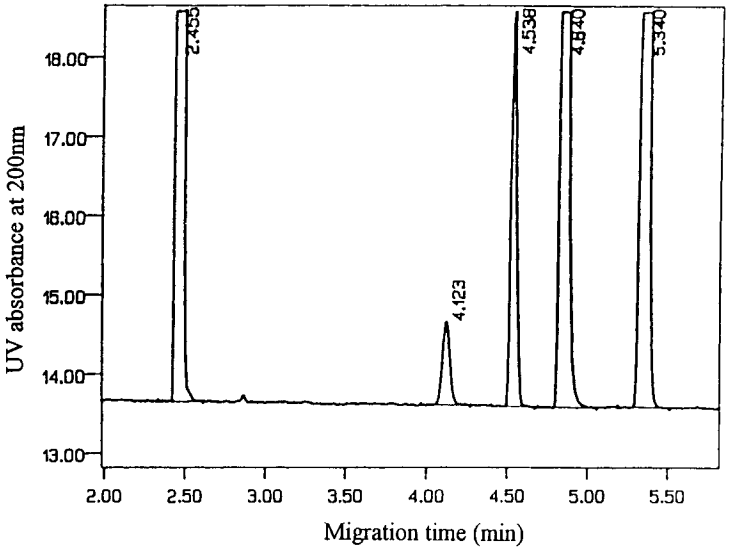

Figure 2. Separation of the multi-drug mixtures used in the extraction procedure optimisation experiments. Separation conditions: as Figure 1. Peak identities : 2.455 min imidazole, 4.123 min. ranitidine, 4.538 min. lamiduvine, 4.840 salbutamol, 5.340 alosetron.

Extraction procedure optimisation

The ability to separate and quantify simultaneously a range of basic drugs is helpful when optimising extraction approaches. For example a volume (eg 100μL) of a standard solution containing a mixture of drugs was spiked onto pre-cleaned surfaces of a known volume and material (for example stainless steel plates 20cm x 20cm). The plates were left to dry and then the drug residues removed by various extraction procedures in order to determine the optimal extraction procedure.

The ability to quantify simultaneously a range of drugs with different solubilities allowed an improved assessment of the extraction procedures rather than use of a single drug. Figure 2 shows a typical separation obtained in these experiments. Imidazole was used as an internal standard. The drug was removed by wiping the surface of the stainless steel plate using a dampened portion of either nylon filter, paper filter or cotton wool.

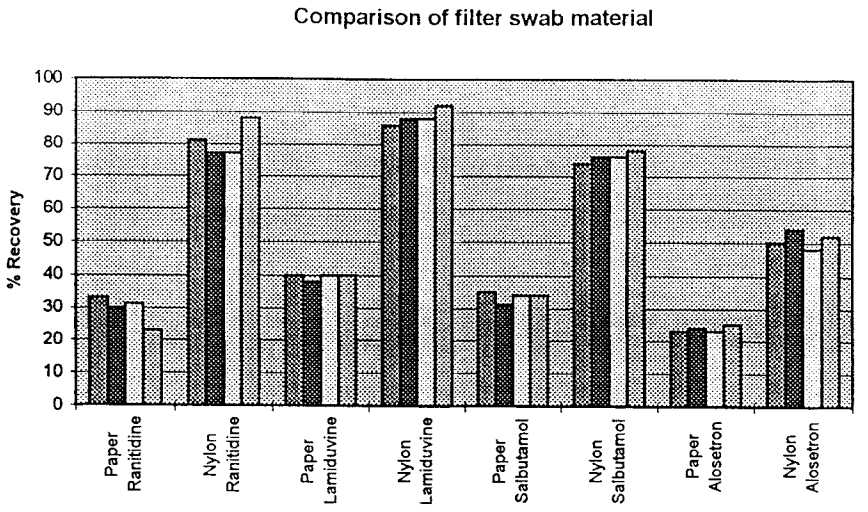

Figure 3. Comparison of the drug recoveries obtained with different swab materials for a range of basic drugs.

Swab material investigation

The impact of the type of material used for the swab was assessed by spiking a range of basic drugs onto plates at either 50 or 250μg levels. The residues were removed by wiping the plates with either nylon filters, paper filters or cotton wool swabs moistened with methanol. The amounts removed was highly dependent upon the swab material used (Figure 3). Nylon was the most effective and cotton wool and paper were similar. The recovery levels showed good repeatability (Figure 3) as 4 extractions were performed to assess precision. It is suggested that the nylon filter is more flexible and has higher coefficient of drag which generates better mechanical removal of the drug compared to the cotton wool or paper. In addition, the level of drug binding onto the nylon filter may be lower than the other alternatives, which would again improved recovery from the swab. Ondansetron gave the lowest recoveries as it is the least water-soluble of the test solutes.

Double-swab technique

An additional experiment involved a double procedure in which cotton wool, heavily wetted with methanol, was used to wipe the surface and a further dry cotton wool swab was used to wipe the wet surface of the plate. The two cotton wool swabs were then independently analysed.

Table 2

Recovery Data for Basic Drugs at Two Concentration Levels Using a "Double Swab" Extraction Procedure

	μg Recovered on Swab			
50μg	**Ranitidine**	**Lamivudine**	**Salbutamol**	**Sumatriptan**
First Swab	24	24	24	24
Second Swab	14	17	16	14
Total	38	41	40	38
% Recovery	76%	83%	80%	76%
250μg	**Ranitidine**	**Lamivudine**	**Salbutamol**	**Sumatriptan**
First Swab	120	116	117	119
Second Swab	70	81	80	69
Total	190	197	197	188
% Recovery	76%	78%	79%	75%

The results (Table 2) clearly indicate the total drug residue extracted was higher using this double-swab technique. The % recovery of total drug was equivalent at the two concentrations investigated. To reduce analysis time it would be possible to extract both swabs together to produce a single solution for analysis.

Acidic Drug Residues

A 10mM borate buffer was found to be suitable for a wide range of water-soluble and water-insoluble acidic drugs. A low voltage 6.5kV in combination with a short (27cm) 100μm capillary enabled a short analysis time (3-5 minutes) with an acceptable level of operating current. Aminobenzoate and β-naphthoxy acetic acid are generally used as internal standards.

Analytical performance

Figure 4 shows a separation for a calibration solution for the determination of an acidic drug (Troglitazone). Use of β-naphthoxy acetic acid as the internal standard enabled sub 5% RSD values to be obtained for calibration solution injection repeatability throughout routine analytical sequences. Linearity for Troglitazone was demonstrated by analysing in

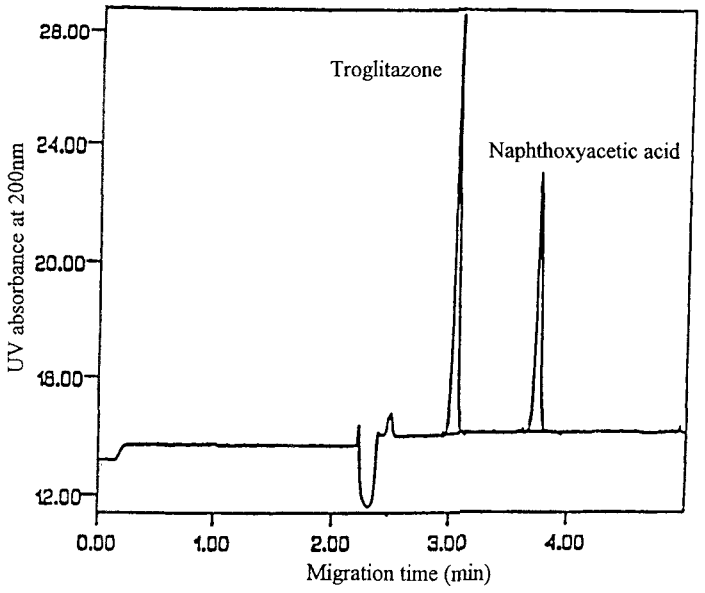

Figure 4. Separation for a Troglitazone solution with the internal standard β-naphthoxy acetic acid. Separation conditions: 27cm x 100μm capillary (20cm to detector), 200nm, 6.5kV, 15mM borate, 30C, sample 0.01μg/mL Troglitazone in 0.02μg/mL standard b-naphthoxy acetic acid (50:50 %v/v Acetonitrile:water).

duplicate five standard solutions covering the range 0.3-30μg/mL. A correlation coefficient of 0.99959 was obtained with an intercept value of -0.6% of the 30μg/mL value. A limit of detection of 0.02μg/mL was obtained which equates to 0.2μg/swab as 10mL of acetonitrile:water (1:1) was used for extraction of the swabs. A limit of quantitation of 0.07μg/mL (0.7μg/swab) was obtained.

The method has also been used to determine trace levels of the antibiotic, cefuroxime. A limit of detection of 0.25μg/mL was obtained with detection at 276nm.

Injection precision for 10 injections of a 10μg/mL solution gave RSD values ranging between 0.6-1.5% using β-naphthoxy acetic acid as the internal standard. Linearity over the range 20μg/mL-2.5μg/mL gave a correlation coefficient of 0.99994.

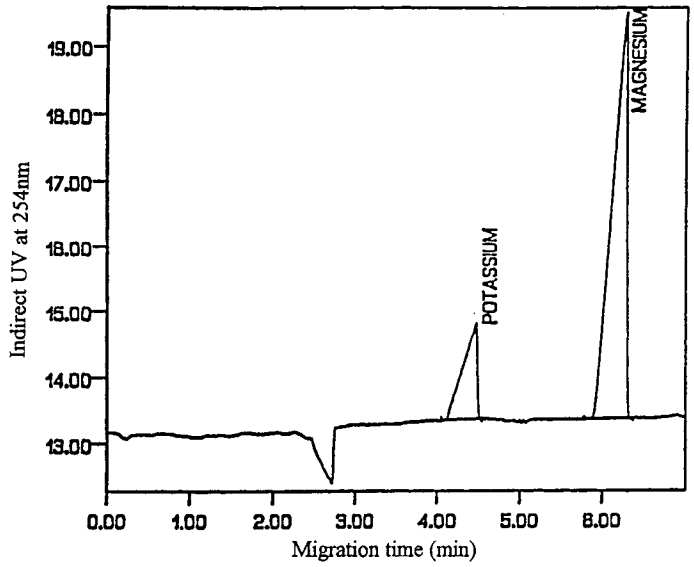

Figure 5. Separation of a potassium with magnesium as an internal standard. Separation conditions: 57cm x 75μm capillary (50cm to detector), indirect UV at 254nm, 20kV, 5mM $CuSO_4$/4mM formic acid/3mm 18-crown-6, 30C, sample 50mg/L potassium in 50mg/magnesium.

Determination of Detergent Residues by Monitoring Potassium Levels

Residues of a caustic detergent solution containing KOH were monitored by determining the levels of potassium by an indirect UV detection CE method. The electrolyte contains formic acid and crown ether to adjust the selectivity and $CuSO_4$ to provide the background UV signal. Magnesium was the preferred internal standard but this would be inappropriate if the product being manufactured, or the detergent solution used, contains levels of magnesium. Figure 5 shows separation of a potassium standard with the magnesium internal standard.

Analytical performance

A sensitivity of 0.5μg/mL was obtained for potassium which was considered an acceptable level. The sensitivity corresponds to 5μg detergent residue/swab. The residue sensitivity was based upon the %w/w of the solid residues corresponding to KOH, and the extraction volume of 5mL of water-based internal standard solution. The sensitivity obtained was considered

adequate as it was an improvement over the titration method previously employed. Linearity for potassium was assessed over the range 1.25-125μg/mL and correlation coefficient of 0.99970 was obtained with an intercept value of 0.4% of the 125μg/mL value.

CONCLUSIONS

The previous use of CE to monitor trace levels of drugs and detergent residues has been considerably extended. A previously reported method for the determination of a wide range of basic drugs has been optimised by use of shorter capillaries and the incorporation of internal standards. The ability to monitor simultaneously a range of drugs with different solubilities was shown to be effective in developing optimised extraction techniques.

A new method was developed for the determination of a range of acidic drug residues. The method was shown to give sufficient accuracy and sensitivity. Trace levels of detergent solution containing KOH have been quantified by monitoring the levels of potassium by CE using indirect UV and magnesium as an internal standard.

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