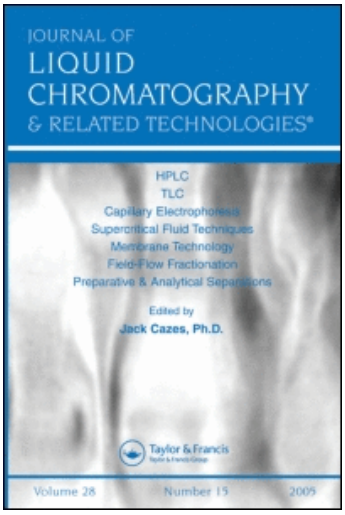

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Validation of a Micellar Electrokinetic Capillary Chromatography (MECC) Method for the Determination of p-Toluenesulfonic Acid Impurity in a Pharmaceutical Intermediate

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VALIDATION OF A MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY (MECC) METHOD FOR THE DETERMINATION OF p-TOLUENESULFONIC ACID IMPURITY IN A PHARMACEUTICAL INTERMEDIATE

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ABSTRACT

Micellar Electrokinetic capillary chromatography (MECC) has been employed for the quantitation of p-toluenesulphonic acid (pTSA) impurity levels in 5-fluoro-3-[3-(1-piperazinyl)-Propyl]-1H-indole (BMS 180317-01) a key intermediate used in the synthesis of a novel antidepressant drug candidate BMS 181101-02. The MECC method demonstrated good selectivity, precision,

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accuracy, linearity, limit of detection and quantitation. MECC of small ionic or polar molecules offers a useful alternative to ion chromatography, ion pair chromatography and RP-HPLC.

INTRODUCTION

In the past several years capillary electrophoresis (CE) has grown rapidly since the work of Jorgenson and Lukacs [1] in the early 1980's. This rapid growth has generated a variety of CE modes applicable to a broad range of charged and neutral species. Micellar electrokinetic capillary chromatography (MECC) was originally introduced in the mid 1980's by Terabe et. al (2) as a way to separate neutral molecules using CE. Terabe later classified the technique as a type of liquid-liquid chromatography without any solid support to hold the stationary liquid phase. Separation is achieved due to the selective partitioning of the ionic and nonionic solutes between the micelles and the surrounding aqueous phase. It was soon realized, that MECC could also be used for the separation of charged compounds [3,4]. In vast majority of all MECC studies sodium dodecylsulfate (SDS) is used as the micelle phase (5). This is primarily due to the fact that SDS is an extremely well behaved and well understood surfactant.

MECC has been shown to be of use for the separation of a range of ionic and nonionic drug classes including antibiotics [6,7] non-steroidal anti-inflammatories [8], steroids [9], analgesics [10] and water soluble vitamins [11].

When analyzing small ionic or polar molecules as impurities in pharmaceutical bulk materials by HPLC, early analyte elution (void volume) and the limited pH operating range of many silica columns are a commonly occurring problem. MECC was found well suited for the separation of the ionic impurity p-toluenesulphonic acid (pTSA) in our case. MECC was analogous to the conventional liquid-liquid partition chromatography producing separation comparable to the HPLC.

As shown in Figure 1, pTSA is a by-product generated in the synthesis of the key intermediate BMS 180317-01. Since the potential of pTSA and its related adduct in the final drug was of great concern, it was rather important to control the residual levels of pTSA at the intermediate step. A quick and reliable method was warranted for the quantitation of pTSA in BMS 180317-01. An HPLC method using a polymer based column and high pH mobile phase was developed with acceptable trace levels detection of pTSA. However, the use of high organic solvent content in the eluent resulted in frequent high back pressure and shorter column life. Establishing a

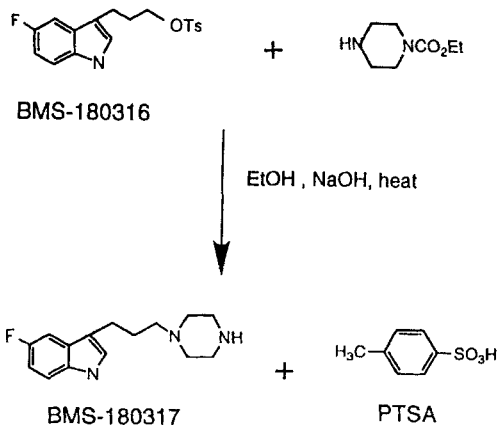

Figure 1 BMS 180317 synthesis scheme

rugged HPLC method may have been possible after extensive method development efforts. However, in this case, suitable MECC operating conditions were quickly identified and then validated for the separation and quantitation of residual pTSA levels in BMS 180317-01. Method validation included measurement of precision, accuracy, linearity, limit of detection and quantitation. The results obtained for several batches of BMS 180317-02 were comparable to those from an HPLC method.

EXPERIMENTAL

Materials and Chemicals

p-Toluenesulfonic acid, monohydrate (pTSA) was purchased from Aldrich chemicals (Milwaukee, WI, USA).

Sodium dodecyl sulfate, sodium borate and acetonitrile were purchased from Fisher Scientific (St. Louis, Mo, USA) and boric acid was purchased from Mallinckrodt (Paris, KY, USA). Water used in preparations of buffers and samples was obtained from a Millipore Milli-Q-System (Bedford, MA, USA). BMS 180317-01 was synthesized by the chemical process technology group at Bristol-Myers Squibb.

Instrumentation and Separation Conditions

A Biorad (Richmond,CA, USA) BioFocus 3000 capillary electrophoresis system was used in all measurements. The fused silica capillaries (50 μm I.D.) used in this study were purchased form Polymicro Technologies (Phoenix, AZ, USA). The separation conditions employed are summarized in Table I.

For optimal performance, the capillaries were preconditioned for 10 minutes at high pressure with 1.0 M NaOH, followed by 2 minutes water and 2 minutes run buffer before the first use. Between injections the capillaries were flushed with run buffer, water, and again run buffer for 20 seconds under high pressure.

Sample Preparation

The choice of an appropriate sample solvent is quite critical in CE since the sample solvent can improve separation efficiency and resolution. The pTSA working

TABLE I

SEPARATION CONDITIONS

System	Biorad Biofocus 3000 Capillary Electrophoresis System
Capillary	Uncoated, 25 cm effective X 50 μm I.D., Temperature 25 degrees
Run Buffer	100 mM boric acid/20 mM sodium borate/20 mM sodium dodecyl sulfate
Voltage	+5 kV, constant
Sample Introduction	8 psi*sec at 5 psi pressure
Detection	190 nm
Sample Concentration	500 ppm
External Standard	PTSA 2% w/w (10 ppm) in BMS 180317 (500 ppm)
Pre-injection Purge	20 seconds: run buffer/water/run buffer

standard (2% w/w) was prepared by spiking 10 ppm pTSA dissolved in 90:10 water:acetonitrile, into 500 ppm BMS 180317 reference standard solution. The stock solution of BMS 180317-01 samples were prepared by dissolving into 90:10 sodium borate (12mM) / boric acid buffer (12mM): acetonitrile to give 1.0 mg/ml concentration and then further diluted with water to 0.5 mg/ml.

RESULTS AND DISCUSSIONS

Optimum conditions for separating pTSA from BMS 180317-01 were obtained with a background electrolyte

containing 100mM boric acid/20mM sodium borate buffer containing 20mM SDS (pH 8.5). Under these conditions the anionic pTSA travels towards the anode; however, the net electroosmotic flow in the direction of the cathode sweeps it through the detector window. A typical separation achieved is given in Figure 2.

The validation undertaken for the MECC method follows the guidelines suggested for HPLC method validation [12].

Linearity

To fully assess the linear dynamic range of the UV detector response at 190 nm, a wide range of samples containing varying amounts of pTSA from 0.02% to 5.0%, relative to the BMS 180317-01 nominal concentration of 500 ppm, were analyzed. Linearity of the MECC method was demonstrated over this range and a correlation coefficient of 0.9999 was obtained for the normalized peak area of pTSA versus the %w/w concentartion of pTSA in BMS-180317. The higher pTSA response at 190nm was found to offset the less than optimum signal to noise ratio when compared with the response at the longer wavelength.

Precision of Peak Area and Migration Time

The precision results are summarized in Table II. A 2% w/w spike solution of pTSA (10 ppm) into BMS 180317-

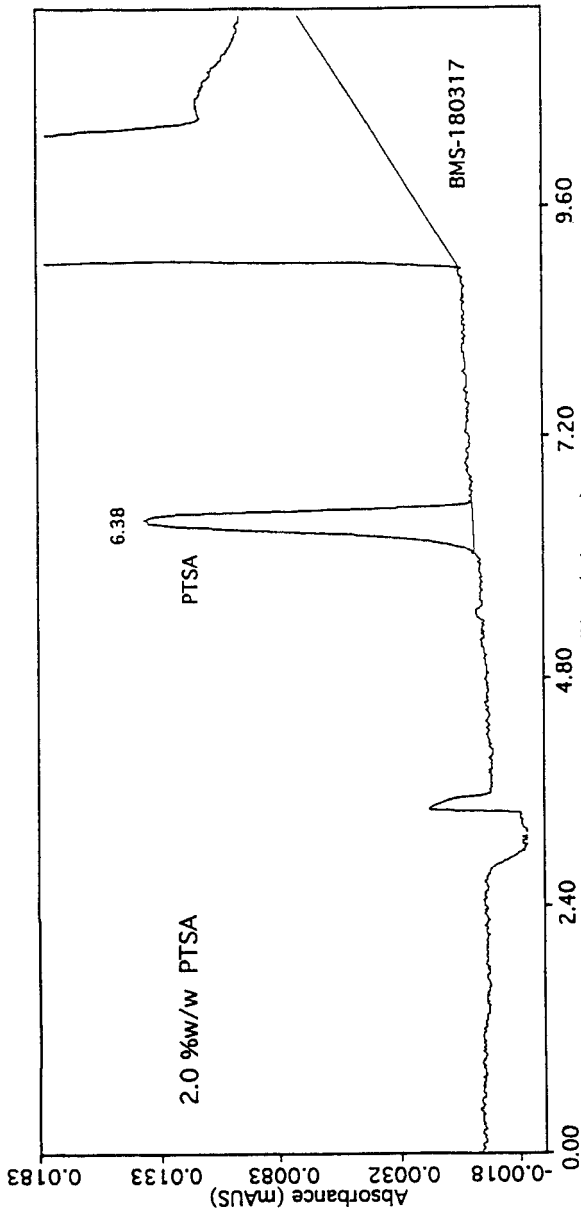

Figure 2 Electropherogram of a BMS 180317 spiked with 2% w/w pTSA. Separation conditions summarized in Table I.

TABLE II

PRECISION OF MIGRATION TIME AND PEAK AREA.

Injection Number	Migration Time (minutes)	Normalized Peak Area
1	6.44	308996
2	6.38	305166
3	6.38	300608
4	6.34	309332
5	6.33	291610
6	6.31	291154
7	6.39	283968
8	6.39	289758
Average	6.37	297574
RSD	0.6%	3%

01 (500 ppm) was injected 8 times and an RSD of 3% was obtained for the pTSA peak area. The RSD on the migration time of the pTSA peak was 0.6%. The area of the pTSA peak was normalized by its migration time in all the calculations to compensate for differences in the residence times in the detector [13].

The repeatability of the analysis for pTSA content was established by analyzing six individual preparations of BMS 180317-01 and calculating the levels of pTSA. The relative standard deviation of these repeat analysis for a sample containing 0.11% w/w pTSA was 7%.

Typical precision data of 0.5 - 2% RSD for peak area using an automated CE instruments have been reported in several publications [14,15]. Equipment manufacturers also typically quote that RSD's of less than 2% can be routinely obtained for peak areas. The less than optimum pricision of 3% for the peak area counts was primarily due to the difficulties associated with the integration and some what poor injection pricision of the Biorad system, precipitation or loss of the sample was not found to be the contributing factor. It is very important to emphasize that for impurities measurement at low levels it is rather difficult to maintain less than 2% RSD for peak areas. The reported values for precision of the method are adequate for its intended purpose of determining low levels of pTSA in BMS 180317-01 intermediate.

Accuracy

The accuracy of the MECC method was established by spike recovery approach. A reference standard of BMS 180317-01 containing no detectable levels of pTSA was spiked to get 0.05, 0.1, 0.2 and 2.0% pTSA relative to BMS 180317-01 (500 ppm). The average recovery was 99% when compared against a pTSA standard. For impurities measurement, this is considered acceptable. The data is summarized in Table III.

TABLE III

ACCURACY (RECOVERY) OF PTSA IN BMS 180317-01 SAMPLES

Spike Level	Recovery
0.05% w/w	104%
0.1% w/w	96%
0.2% w/w	96%
2.0% w/w	101%

Average Recovery 99.0%

Sensitivity

The sensitivity of the method in terms of limit of detection (LOD signal to noise ratio greater than 3) and minimum quantifiable level (MQL, signal to noise ratio greater than 10) was evaluated. The method was found to have an limit of detection of 0.02% (w/w) and a limit of quantitation of 0.08% (w/w). The LOD and MQL were determined by measuring the standard deviation of the peak-to-peak noise of the system using solvent blank. These low levels of LOD are comparable to levels reported by Altria for dimeric impurities present in salbutamol drug substance using low UV wavelength detection [16].

Comparison with HPLC

pTSA content determined by MECC for several batches of BMS 180317-01 was compared with the values obtained by

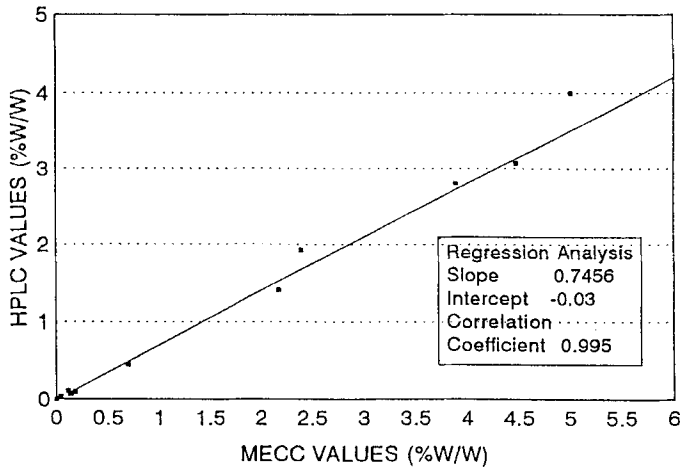

Figure 3 Comparative analysis by HPLC and MECC of pTSA levels in BMS 180317 samples.

a validated reverse phase HPLC method. Figure 3 shows the correlation between the two techniques. Although the HPLC method was found to give slightly higher results as indicative from the slope of the correlation line, the correlation coefficient of 0.995 was indicative of the overal agreement between the two methods.The small differences in results between the two methods at high levels could very well be due to the sample preparation and differences in the peak integration software packages used. Although the HPLC method was equally acceptable, MECC method was found to be faster, economical and rugged.

CONCLUSION

A MECC method was validated for the quantitative determination of p-toluenesulphonic acid impurity in BMS 180317-01, a pharmaceutical intermediate. Method validation demonstrated acceptable levels of performance in terms of precision, sensitivity, reproducibility and accuracy. The MECC method offered an useful alternative to the HPLC method and was demonstrated to give similar levels of method performance and comparable results. Although the MECC approach gave acceptable separation, the potential of free solution CE (FSCE) needs to be evaluated and it is possible that similar or better separation can very well be achieved with a simple FSCE method.

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