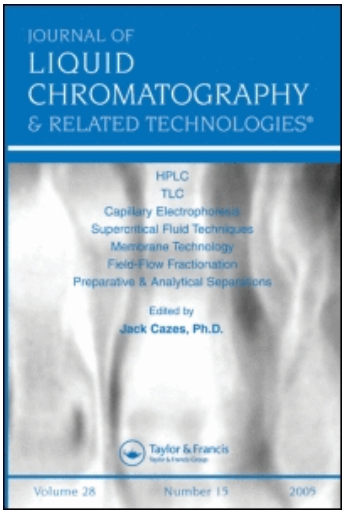

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Sensitive Micellar Electrokinetic Chromatographic Determination of Salbutamol, Guaifenesin, and Dyphylline in Oral Formulations

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Abstract: A sensitive micellar electrokinetic chromatographic method to analyze salbutamol, guaifenesin, and dyphylline employing 20 μm inner diameter capillaries was developed. Sensitive analysis of the drugs at a concentration range of about 0.5 to 60 μg/mL was demonstrated using large volume sample stacking with 75% acetonitrile as the sample diluent. Using dyphylline as the internal standard, detection limits were 0.07 μg/mL and 0.21 μg/mL for salbutamol and guaifenesin, respectively. Using the standard addition method, accurate determination of salbutamol and guaifenesin in syrup and capsule preparations was achieved. Recovery values were satisfactory (95 to 100%), but matrix interferences affected the reproducibility.

Keywords: Acetonitrile stacking, Dyphylline, Guaifenesin, Large volume sample stacking, Micellar electrokinetic chromatography, Salbutamol

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INTRODUCTION

The development of modern high performance capillary electrophoresis (HPCE) can be traced back in 1981, when Jorgenson and Lukacs[1] published a pioneering work by using a capillary with 75 μm inner diameter (i.d.) to obtain efficient separations of amino acids, dipeptides, and amines. In comparison to 200 μm or 300 μm i.d. capillaries used in earlier studies by Hjertén,[2] Virtanen,[3] and Mikkers et al.,[4] this narrower capillary has a higher surface area to volume ratio that allows efficient dispersion of the heat generated from the applied high voltage. With years of research and development on HPCE, a commercial automated CE instrument was introduced in the late 1980s and CE started to become a more popular separation technique. Capillaries of 25 μm to 75 μm i.d. are commonly used in this modern instrument.

In the pharmaceutical industry, the application of CE for the separation of drug enantiomers has been established as a very efficient technique because of its high efficiency, short analysis time, versatility due to the great variety of chiral selectors that can be added to the background electrolyte, short equilibration times required when changing the chiral selector, and low consumption of selectors.[5–10] In our previous work,[11] enhanced enantioresolution and faster analysis times were achieved with 20 μm i.d. capillaries in comparison with the 50 μm i.d. for the enantioseparation of 10 basic drugs with β-cyclodextrin as chiral selector. In another study,[12] the poor sensitivity of these narrower capillaries was overcome by applying large volume sample stacking. In particular, the analytes diluted in aqueous solution of high acetonitrile (ACN) concentration were loaded either by hydrodynamic or electrokinetic injection at longer intervals without polarity switching and electro-osmotic flow (EOF) manipulation. With the simple method described, about several hundred- and a thousand-fold sensitivity enhancement was achieved with hydrodynamic and electrokinetic injections, respectively. The 20 μm i.d. capillary was also used for investigating the effects of alcohols on the enantioseparation of selected basic drugs with native γ-CD.[13] Results obtained from the binding studies and the relatively high correlation coefficients obtained between resolution vs. log P and between resolution vs. ovality (i.e., parameter to indicate bulkiness of a molecule) confirmed that hydrophobicity and/or bulkiness of alcohols have an influence on the enantioresolution of most of the analytes studied.

Micellar electrokinetic chromatography (MEKC) is a separation technique for charged and non-charged species, which was developed by Terabe and coworkers.[14,15] The instrumentation used to carry out this technique is identical to that in capillary electrophoresis, and capillaries of 50 μm to 75 μm i.d. are usually employed. This technique has a similar scope with that of reversed phase high performance liquid

chromatography (RP-HPLC), having advantages over HPLC with regard to the efficiency of the separation system, separation speed, cost, and tolerance to matrix constituents. In MEKC, an ionic surfactant is used as a pseudostationary phase that corresponds to the stationary phase in conventional chromatography and the surrounding aqueous phase to the mobile phase. The separation principle of analytes is based on their differential partitioning between the aqueous phase and the micelle phase.

Relatively low detection sensitivity is one perceived limitation of MEKC when compared to HPLC, but online sample preconcentration methods, such as sample stacking and sweeping, are now available. A review by Kim and Terabe[16] gives a comprehensive discussion on a variety of sample stacking and sweeping modes and their applications.

This study aims to develop a simple sensitive MEKC method employing 20 μm i.d. capillaries. For this purpose, guaifenesin (GG), dyphylline (DYP), and salbutamol (SAL) were chosen as the analytes. GG, (R,S)-3-(2-methoxyphenoxy)propane-1,2-diol, is one of the most widely used expectorants present in a variety of pharmaceutical formulations.[17,18] DYP, (7-(2,3-dihydroxypropyl)-1,3-dimethylpurine-2,6-dione), a xanthine derivative, is a bronchodilator used for the relief of acute bronchial asthma and for reversible bronchospasm associated with chronic bronchitis and emphysema. SAL, (2-(hydroxymethyl)-4-[1-hydroxy-2-(tert-butylamino)ethyl]-phenol), on the other hand, is a β_2-selective adrenoreceptor agonist, which actuates as a pronounced bronchodilatory, cardiac, uterine, and metabolic agent. It is administered in a variety of ways, including tablets or syrup, inhalation by aerosol, and injection. It is normally used in the sulfate form. Oral formulations of GG in combination with DYP (brand names: Dilor-G, Dyflex-G, Dyphyllin-GG, Lufyllin-GG, Panfil-G, etc.) or with SAL (brand names: Asmalin Broncho, Broncaire Expectorant, Clarituss Plus, Pulmovent, Salvex XP, Ventar Expectorant, Ventolin Expectorant, Venzapril, etc.) are widely available in the market.

In this study, optimization of stacking and separation conditions for a sensitive and reliable analysis of the drugs specified using 20 μm i.d. capillaries, comparison of conditions, and results between 20 μm and 50 μm i.d. capillaries, and application of the optimized method with 20 μm i.d. capillaries to determine drug content in oral preparations are presented.

EXPERIMENTAL

Apparatus

Capillary electrophoretic stacking and separation were performed with HP^{3D} CE system Model G1600A (Hewlett-Packard, Waldron, Germany)

equipped with a diode array detector and ChemStation software for data analysis. Uncoated fused silica capillaries of 50 μm i.d. (375 μm o.d.) and 20 μm i.d. (375 μm o.d.) × 48.5 cm (40 cm effective length) were purchased from GL Science (Tokyo, Japan). The pH was adjusted using pH meter HM-30 V (TOA Electrochemical, Japan).

Reagents and Materials

Sodium hydroxide, sodium borate, sodium dodecyl sulfate (SDS), and triethylamine (TEA) were obtained from Kishida Chemical (Osaka, Japan). Methanol was purchased from Nacalai Tesque (Kyoto, Japan), while GG and ACN were from Wako Pure Chemical (Osaka, Japan). SAL and DYP were obtained from Sigma-Aldrich (Tokyo, Japan). Water was purified with a Milli-Q system Millipore (Tokyo, Japan). A 0.2 μm disposable nylon filter was obtained from Toyo Roshi Kaisha (Tokyo, Japan). Commercial preparations of GG/SAL syrup (50 mg/L 5 mg/mL) and capsule (100 mg/2 mg/cap) were purchased from a drugstore in the Philippines.

Buffer and Sample Preparation

Borate buffer was prepared from 60 mM sodium borate solution, which was diluted with water to the desired concentration and adjusted to the desired pH with 1.0 M sodium hydroxide. An appropriate amount of SDS was weighed and dissolved in the buffer by mixing, using a magnetic stirrer. Methanolic stock solutions (1 mg/mL) of each analyte were prepared and subsequently, diluted to the desired concentration with the specified matrix (i.e., aqueous ACN). All solutions were filtered through 0.2 μm filters prior to use.

The syrup samples were prepared by taking 100 μL of the syrup diluted with water, to make a stock solution equivalent to 1.0 mg/0.02 mg/mL GG/SS, and filtered through 0.2 μm filters. From this solution, 20 μL was taken and a specified volume of standard solutions were added and diluted with water and ACN, such that the final concentration of ACN in the sample would be 75%. The resulting solution was then analyzed.

Samples from capsules were prepared by determining the weight of the pooled contents of 5 gelatin capsules and subsequently, triturated using a mortar and pestle to achieve uniform distribution of the drugs to the excipient. The weight of an amount equivalent to about 1.0 mg/0.02 mg/mL GG/SS was then taken, dissolved in deionized water, and filtered. From this filtered solution, 20 μL was taken and a specified volume of standard solutions were added and diluted with water

and ACN, such that the final concentration of ACN in the sample would be 75%. The resulting solution was analyzed subsequently.

CE Operation

Newly installed capillaries were flushed with 0.1 M sodium hydroxide (20 min), then by deionized water (20 min), and finally with the buffer (20 min) at 30°C. At the start of the experiment each day, capillaries were washed with 0.1 M sodium hydroxide, deionized water, and running buffer for 5 min each. To ensure repeatability, capillaries were flushed with 0.1 M sodium hydroxide (3 min), followed by deionized water (3 min), and then with the running buffer (5 min) in between runs. The capillary temperature was maintained at 25°C throughout the analysis. Samples were injected at the anodic end hydrodynamically, using the flush mode (about 935 mbar) at different intervals.

For the 20 μm i.d. capillaries, a 1.0 cm injection plug length requires the application of 50 mbar for 80 s. In order to significantly decrease the time required during injection, application of higher pressure by means of the flush mode can be done.[12] Voltage, 30 kV for 20 μm, while 15 kV for 50 μm i.d. capillaries, was applied at positive polarity with detection at the cathodic end. Detection was performed at 200 nm.

RESULTS AND DISCUSSION

Optimization of Separation Conditions

The effects of various parameters were initially investigated to determine the optimum conditions applicable for the set of drugs being studied. The effect of pH varied from 9.4 to 10.2 is shown in Figure 1. At pH 9.4, SAL and DYP comigrated, while GG was detected as a separate peak. At pH 9.8, improvement in the separation of SAL and DYP was observed without any negative influence on the GG peak. However, increasing the pH to 10.2 resulted in the comigration of SAL and DYP. Hence, pH 9.8 was chosen as the optimum pH.

Upon changing the concentration of SDS to either 20 mM or 30 mM, the resolution achieved between SAL and DYP peaks deteriorated, implying that 25 mM SDS is most favorable for the separation. Increasing the buffer concentration to 60 mM allowed for a satisfactory separation of the 3 analytes (Figure 2a).

In a previous study,[19] wherein the commonly used 50 μm i.d. capillary was employed and borate buffer as one of the buffer systems for the determination of SAL in syrups was utilized, SAL migrated with

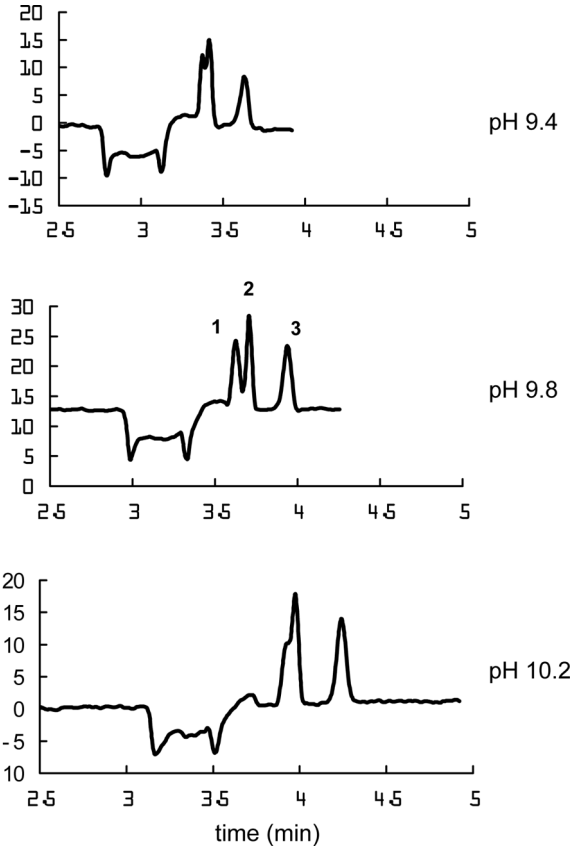

Figure 1. Effect of pH on separation of SAL (1), DYP (2), and GG (3). Other conditions: capillary 20 μm i.d. × 48 cm (40 cm effective length), 30 mM borate buffer with 25 mM SDS, capillary temp. 25°C, applied voltage 30 kV, detection at 200 nm, flush injection for 0.25 min, analytes in 75% ACN, SAL and GG 20 μg/mL, DYP 30 μg/mL.

the EOF when the BGE contained 5 mM borate buffer pH 9.0 without SDS and the applied voltage was 25 kV. When it was necessary to increase the buffer concentration to 30 mM at pH 9.4 and add 50 mM SDS, it became essential to decrease the voltage to 15 kV because of the higher conductivity of the electrolyte. As a consequence of decreasing the voltage, analysis time was significantly increased.

In this study, the current generated was only about 35 μA when a voltage of 30 kV was applied on a run with BGE containing 60 mM borate buffer and 25 mM SDS with the 20 μm i.d. capillary. The use of lower voltage did not result in any improvement of the separation but

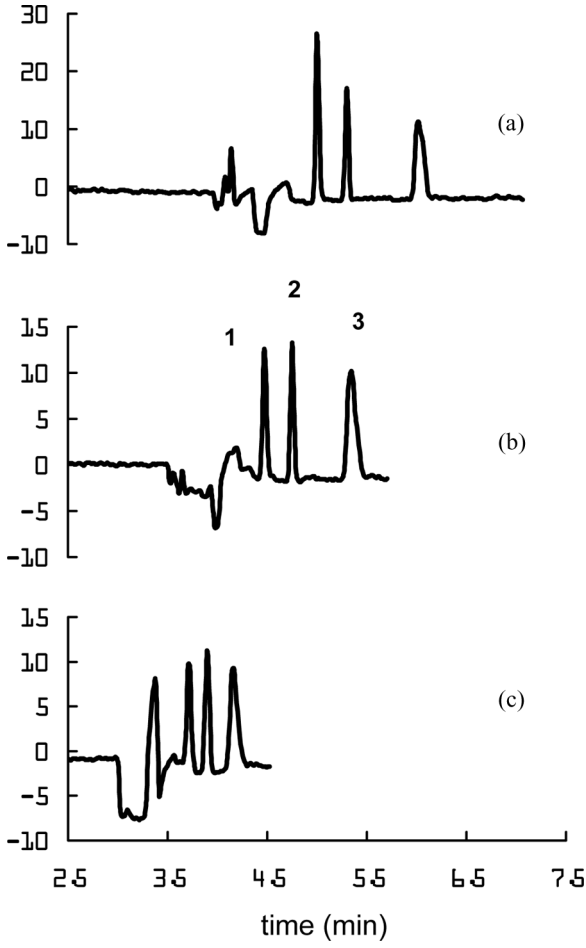

Figure 2. Effect of ACN as sample diluent on stacking and separation of SAL (1), DYP (2), and GG (3). (a) 75% ACN, (b) 70% ACN, (c) 65% ACN. 60 mM borate buffer pH 9.8. Other conditions are the same as in Figure 1.

only increased the analysis time. Thus, 30 kV was used in all the analyses that employ these narrower capillaries.

Effect of Sample Matrix on Stacking and Separation

Various online preconcentration techniques are available and one of the simplest among them is large volume sample stacking.[20] Previous studies showed that the use of high ACN concentration in the sample diluent is

favorable for stacking because of the much lower conductivity that it could impart compared to diluting the samples in water only.[12,21] The lower conductivity in the sample zone allows for the charged analytes to move more rapidly towards the separation zone in which better stacking could be achieved.

In this study, ACN concentration in the sample diluent was varied from 60 to 80%. At the same time, the injection time interval was varied to determine the longest injection time that would not give detrimental effects on the separation, i.e., the occurrence of voltage drop across the separation zone. It was found that the optimum injection time via flush mode is 0.25 min, which is equivalent to about 9% capillary volume.

In Figure 2, the effect of ACN concentration on stacking and separation is shown. From the figure, better separation accompanied by an increase in the analysis time can be observed with increasing amounts of ACN. It should also be noted that when ACN added was less than 50%, the analytes migrated faster and were detected as a single sharp peak. From Figure 2a, where ACN concentration = 75%, best results were achieved in which the peak height of salbutamol increased by 2 times when compared to those obtained with other ACN concentrations. However, minimal increase in peak height was observed with both DYP and GG. SAL is usually administered at relatively low doses because it can produce a certain amount of anabolic-like effects depending on the route of administration.[22] Also, in drug formulations of SAL-GG combination, it is usually present at a much lower concentration. Hence, better sensitivity that can be obtained for this drug is highly beneficial for its analysis in combination with GG or other drugs.

Capillaries, 20 μm i.d. vs. 50 μm i.d

The stacking and separation conditions using a 50 μm i.d. capillary was optimized. Similar conditions were obtained except for some differences. First, the applied voltage must be limited to 15 kV because of the high current generated (up to 100 μA) during the run. Second, the injection time using the flush mode was reduced to 0.03 min to prevent deleterious effects on separation due to overloading. Third, the highest ACN concentration in the sample diluent was limited to only 70%. At higher ACN concentrations, there was a significant voltage drop across the sample zone that caused the field strength across the separation zone to approach zero, and this led to separation failure.

A typical electropherogram for the separation of the three target drugs with the 50 μm i.d. capillary using the aforementioned conditions is shown in Figure 3. From the figure, the analysis time was about 11 min, which is almost twice as long than when the stacking and separation conditions with

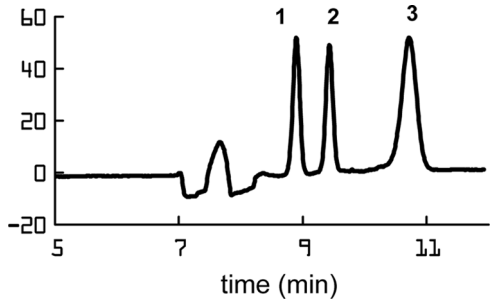

Figure 3. Electropherogram for the separation of SAL (1), DYP (2), and GG (3) using 50 μm i.d. capillary. Applied voltage 15 kV, flush injection for 0.03 min, analytes 25 μg/mL in 70% ACN, 60 mM borate buffer pH 9.8. Other conditions are the same as in Figure 1.

the 20 μm i.d. capillary were applied (Figure 2a). The higher peak signals obtained with the 50 μm i.d. capillary is expected because of its longer path length.

Effect of Triethylamine on Separation and Linearity

Using the conditions described in Figure 2a, calibration curves (in terms of peak area) were constructed for SAL and GG with DYP as the internal standard (consistently added at 30 μg/mL). Although acceptable linear relationship between concentration and peak area was obtained with GG ($r^2 = 0.9807$) at a concentration range of 10–50 μg/mL, poor linearity was obtained with salbutamol ($r^2 = 0.9152$) at the same concentration range. This relatively low r^2 value for salbutamol could be attributed to the interaction of the cationic salbutamol ($pKa_1 = 9.3$, $pKa_2 = 10.3$) to the underivatized silanol groups of the capillary wall. This interaction is made more pronounced due to the larger surface area to volume ratio as the diameter of capillaries decrease. TEA, a small aliphatic amine usually used to overcome such a problem, was added to the background electrolyte at varying concentrations (0.2% to 0.75%). The effect of TEA concentration is shown in Figure 4. TEA at 0.2% did not impart any negative influence on both stacking and separation. However, increasing amounts of TEA resulted in increased EOF, affecting the separation, especially, that of the resolution between DYP and GG. Hence, the amount of TEA added to the background electrolyte must be limited to 0.2%.

With 0.2% TEA, it was observed that the peaks of both GG and DYP improved (Figure 2a vs. Figure 4a), wherein the number of theoretical

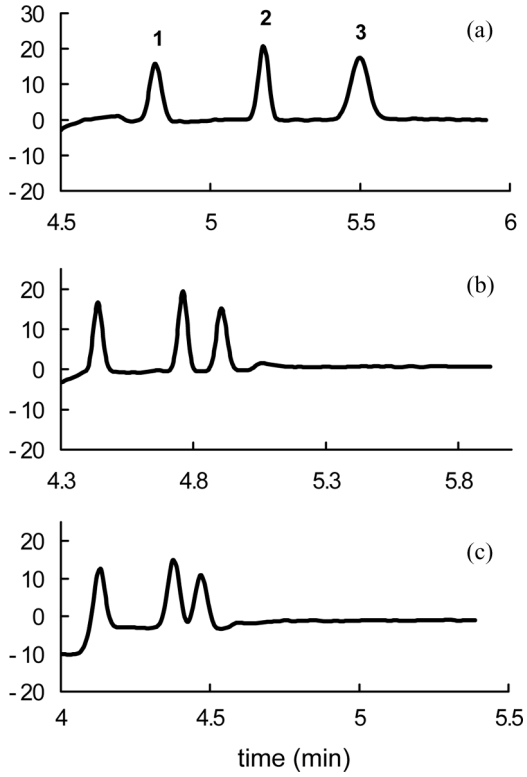

Figure 4. Effect of TEA on separation of SAL (1), DYP (2), and GG (3). (a) 0.2% TEA, (b) 0.35% TEA, (c) 0.75% TEA. 60 mM borate buffer pH 9.8. Other conditions are the same as in Figure 1.

plates increased from 20500 to 32800 for GG and from 63900 to 73800 for DYP. In Figure 2a, it is quite unusual that SAL, which was found to interact with the capillary wall, appeared to have a better peak shape than that of GG. This could be due to the favorable effect of the 75% ACN in the sample diluent on the stacking of SAL while imparting minimal influence on the stacking of the GG peak.

Calibration curves for SAL and GG, in the presence of 0.2% TEA, were then constructed. A comparison of the data obtained for the calibration curves and correlation coefficient in the absence and presence of TEA are summarized in Table 1. An improvement in the linear concentration range and r^2 values was observed for both drugs. This signifies that 0.2% TEA is effective to minimize capillary wall adsorption of SAL, while imparting a favorable effect on both the peak shapes of GG and DYP.

Table 1. Comparison of data obtained for the calibration curves and correlation coefficients of SAL and GG in the absence and presence of TEA

	SAL		GG	
	w/o TEA	with 0.2% TEA	w/o TEA	with 0.2% TEA
Calibration curve equation[a]	$y = 0.0661x + 0.3763$	$y = 0.0463x + 0.0394$	$y = 0.0563x - 0.2011$	$y = 0.0480x + 0.3098$
Correlation coefficient (R^2)	0.915	0.999	0.981	0.998
Linear concentration range (μg/mL)	10–50	1–60	10 – 50	1 – 60

[a]n = 3, constructed in terms of corrected peak area with 7 concentrations 1–70 μg/mL; Other conditions used were the same as in Figure 2a; corrected peak area = (peak area/migration time)$_{\text{Analyte}}$/(peak area/migration time)$_{\text{Internal Standard}}$.

Accuracy, Precision, Detection Limits, and Quantitation Limits

Accuracy of the improved method was determined for both intra-day (repeatability) and inter-day (intermediate precision) variations. Standards of SAL and GG were analyzed at 20 μg/mL. The precision of the method was calculated by analyzing 3 standard samples in 1 day to determine the intra-day variability and on 3 consecutive days to determine inter-day variation. Accuracy was calculated as the percentage of the nominal concentration.

The accuracy and precision data obtained are summarized in Table 2. RSD values for the intra- and inter-day measurements were not more than 1.0 and 0.2%, respectively, indicating good intra- and inter-day precision. High accuracy values for both the intra-day (99.5–100.2%)

Table 2. Accuracy and precision data for the determination of SAL and GG

	Intra-day variations (n = 3)			Inter-day variations (n = 3)		
	Measured[a] (μg/mL)	RSD[b] (%)	Accuracy[c] (%)	Measured[a] (μg/mL)	RSD[b] (%)	Accuracy[c] (%)
SAL	20.04 ± 0.01	0.61	100.2	20.02 ± 0.04	0.18	100.1
GG	19.90 ± 0.01	0.99	99.5	19.91 ± 0.03	0.13	99.6

[a]Values are the mean values ± standard deviation; Concentration of control samples = 20 μg/mL.
[b]Relative standard deviation.
[c]Accuracy = (Mean measured value/Nominal value) × 100.

and the inter-day (99.6–100.1%) measurements were also obtained for the validated method.

The limits of detection (LOD) and limits of quantitation (LOQ) of both drugs were also determined for the improved method. Using $LOD = 3.3\ \sigma/S$ and $LOQ = 10\ \sigma/S$, where σ is the standard deviation of the response of the blank and S is the slope of the calibration curve, the LOD and LOQ for SAL were 0.07 μg/mL and 0.22 μg/mL, respectively. For GG, LOD and LOQ were 0.21 μg/mL and 0.42 μg/mL, respectively.

Determination of the Analytes in Pharmaceutical Preparations

In order to evaluate the applicability of the developed method to pharmaceutical preparations, commercial oral preparations of SAL-GG combination (syrup and capsule) were analyzed. A typical electropherogram obtained for the separation of the analytes in syrup and capsule is shown in Figure 5. Longer migration times of the peaks were evident with syrup and capsules in comparison to that of the standard sample

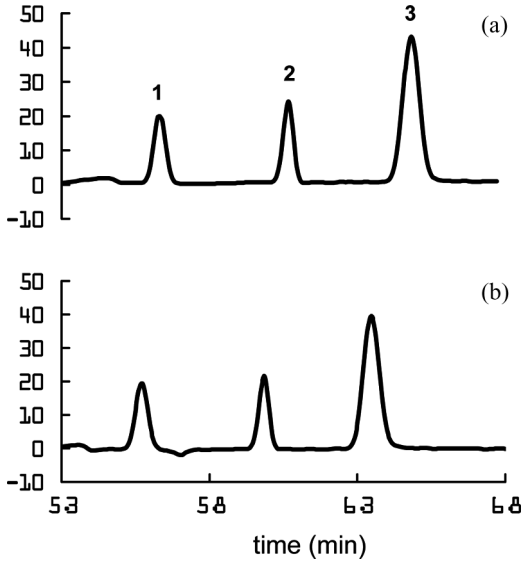

Figure 5. Typical electropherogram for the analysis of SAL (1) and GG (3) in capsule (a) and syrup (b) with DYP (2) as internal standard. Conditions: 60 mM borate buffer pH 9.8 with 0.2% TEA, 20 μL of syrup and capsule samples (both 1 mg/0.02 mg/mL GG/SAL) both added with 20 μg/mL each of GG and SAL standard solutions. Other conditions are the same as in Figure 1.

Table 3. Calibration curve equations and correlation coefficients obtained for the analytes by standard addition method

	SAL[a]		GG[b]	
	Calibration curve equation[c]	Correlation coefficient (R^2)	Calibration curve equation[d]	Correlation coefficient (R^2)
Syrup	$y = 0.0618x + 0.0209$	0.953	$y = 0.0634x + 1.6230$	0.964
Capsule	$y = 0.0877x + 0.0349$	0.995	$y = 0.0915x + 1.8352$	0.984

[a]n = 4, concentration range of standard salbutamol added = 0–4 μg/mL.
[b]n = 4, concentration range of standard guaifenesin added = 0–20 μg/mL.
[c]*versus* calibration curve equation by external standard method $y = 0.0463x + 0.0394$ from Table 1.
[d]*versus* calibration curve equation by external standard method $y = 0.0480x + 0.3098$ from Table 1.

(Figure 4a). Since the samples analyzed were not subjected to any preliminary cleanup procedure and were just merely diluted and filtered, it is possible that the other neutral components in syrup (i.e., preservatives such as parabens and sweeteners such as saccharin) and in capsule (i.e., excipients such as lactose) had an influence in either the EOF or the differential partitioning behavior of DYP or GG, which are both neutral compounds. Thus, prior to quantitative analysis, the presence or absence of matrix interferences was studied. For this purpose, the standard addition method was performed for both syrup and capsule and the data obtained for the calibration curves are shown in Table 3. In comparison to the slope of the calibration curve obtained previously using the external standard method (Table 1), the slopes of the line for both drugs increased by a factor of at least 1.3 for the syrup and at least

Table 4. Determination of SAL and GG in capsule and syrup by the optimized conditions using standard addition method

	SAL		GG	
	Expected amount (μg/mL)	Measured amount (μg/mL)	Expected amount (μg/mL)	Measured amount (μg/mL)
Capsule	0.400	0.398[a] (99.4%)[b]	20.00	20.06 (100.3%)
Syrup	0.400	0.388 (98.8%)	20.00	20.09 (100.4%)

[a]Average value, n = 3.
[b]Values in parentheses are the accuracy of the measurement expressed in %.

Table 5. Recovery data obtained for SAL and GG from the spiked drug preparations

Drug preparation	Drug	Determined amount before spiking (μg/mL)[a]	Spiked amount (μg/mL)	Recovery (%)[b]	RSD (%)
Syrup	SAL	0.388	0.400	95.6 ± 7.7	8.1
	GG	20.09	20.00	99.7 ± 6.7	6.7
Capsule	SAL	0.398	0.400	94.7 ± 7.6	10.1
	GG	20.06	20.00	95.0 ± 10.2	10.7

[a]Calculated from Table 4.
[b]Average value ± standard deviation, n = 3.

1.9 for the capsule. These changes signify the presence of matrix interferences. Hence, the standard addition method was used to determine the drugs in these preparations.

Table 4 summarizes the data obtained for the quantitative analysis of these drugs in the said oral preparations. High accuracy values of the measured amounts were achieved for both drugs in either preparation (98.8 to 100.4%). In Table 5, recovery values obtained from the standard additions of the analytes to the syrup and capsule formulations were satisfactory (94.7 to 99.7%). However, relatively high RSD values were obtained (6.7 to 10.7%) that could be attributed to the matrix interferences observed.

CONCLUSIONS

An accurate, precise, and sensitive MEKC method for the analysis of SAL, GG, and DYP employing 20 μm i.d. capillaries was presented. Compared to the commonly used 50 μm i.d. capillaries, faster separation (6 min vs. 12 min) was achieved with these narrower capillaries due to the more efficient heat dissipation that allows the use of higher voltages (up to 30 kV), even at high buffer conductivity. The simple large volume sample stacking method with the aid of 75% ACN as sample diluent was effective enough to achieve sensitive detection of the drugs, which could be analyzed from about 0.5 to 60 μg/mL concentration range. The addition of very low amounts of TEA (0.2%) to the background electrolyte was necessary to minimize capillary wall adsorption of SAL. Although matrix interferences were evident, the accurate analysis of the drugs in syrup and capsule preparations was demonstrated by the standard addition method. Based on the recovery data obtained, quantitative aspects of the method must be improved. More often than not, high conductivity

buffers are used for MEKC analyses, and in such cases, the advantage that could be obtained with these narrower capillaries provides another option to chromatographers, since the limitations of these capillaries such as wall adsorption and lower sensitivity can be overcome by simple techniques such as the one presented in this study.

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