

# Supercritical Fluid Extraction of Imidazole Drugs from Cosmetic and Pharmaceutical Products

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## ABSTRACT

An effective method for isolating common imidazole drugs clotrimazole (CT) and ketoconazole (KCZ) from cosmetic and pharmaceutical products based on supercritical fluid extraction (SFE) was developed. Creams, tablets and cosmetic shampoos underwent supercritical carbon dioxide fluid extraction and CT and KCZ were determined by reverse-phase high performance liquid chromatography. All supercritical fluid extractions were performed with a 10-min static step, followed by a 15 min dynamic step using acetonitrile as the modifier. After extraction by SFE, KCZ and CT were separated on a C<sub>18</sub> column using an isocratic mobile phase that consisted of methanol/water/diethylamine/glacial acetic acid (80:20:0.3:0.2, v/v, pH 7.0) at a flow rate of 1.0 mL/min. The eluates from column were monitored by absorbance at 224 nm using a diode array detector.

This developed approach was validated by assaying commercial pharmaceuticals for the routine quantification of CT and KCZ in various formulations. The calibration curves for CT and KCZ were linear over the ranges of 50–250 µg/mL and 25–250 µg/mL, respectively. The detection limits of CT and KCZ were 2.31 µg/mL and 0.42 µg/mL, respectively, and the limits of quantitation were 7.70 µg/mL and 1.40 µg/mL, respectively. These drugs contents were found to be in good agreement with the label claim values. Satisfactory extraction recoveries ranging from 90.8% to 97.2% were obtained from five replicate extractions.

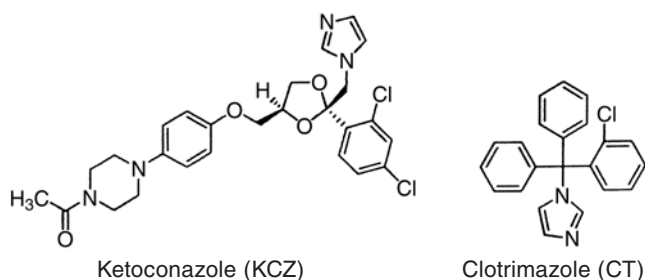
Key words: supercritical fluid extraction, clotrimazole (CT), ketoconazole (KCZ), imidazole drugs

## INTRODUCTION

Clotrimazole (1-( $\alpha$ -2-chlorotrityl) imidazole, CT) and Ketoconazole (*cis*-1-acetyl-4[4-[2-(2,4-dichlorophenyl)-2-(1*H*-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxyphenyl]piperazine, KCZ) (Figure 1) are common broad-spectrum antifungal agents in commercial pharmaceutical preparations. CT, an inhibitor of trout cytochrome P450s<sup>(1)</sup>, is recognized to have neuroprotective effects<sup>(2)</sup> and can be used to treat cancer<sup>(3)</sup>. Occupational contact sensitization in CT by patch test (5% CT in white vaseline) was reported in 1998<sup>(4)</sup>. KCZ is a potent inhibitor of P-glycoprotein and suppresses

the oxidative activity of cytochrome P450 3A4 in the liver. The recommended daily oral dose of KCZ should not exceed 200 mg because of the risk of hepatotoxicity<sup>(5)</sup>. KCZ is authorized for use in antidandruff shampoos at concentrations between 1% and 2%. Therefore, the quantity control of KCZ and CT in pharmaceuticals has been the focus of many researches.

A number of methodologies have been proposed for the analysis of KCZ and CT in pharmaceutical preparations or their subsequent residues in biological fluids. These approaches include adsorptive stripping voltammetry<sup>(6)</sup>, liquid chromatography coupled with tandem mass spectrometry (LC-MS)<sup>(7)</sup>, capillary zone electrophoresis (CZE)<sup>(8)</sup>, spectrophotometric method<sup>(9)</sup>, spectrofluorimetric method<sup>(10)</sup> and reverse-phase high-performance liquid chromatography (HPLC)<sup>(11-13)</sup>, where the HPLC method is the most widely used one. The HPLC method is more sensitive than the CZE method and provides a reproducible quantitation of KCZ in drug matrices (tablets and creams) with a relative standard deviation (R.S.D.) below 1.5%<sup>(14)</sup>. The HPLC method has limited separation efficiency for molecules with high molecular weight (such as KCZ and CT) and this could be improved by employing higher pressure or longer retention time. For example, CT and its analogues were successfully quantified by HPLC with a longer retention time of 30 min<sup>(15)</sup>. Among the published HPLC meth-



**Figure 1.** Chemical structures of Ketoconazole (KCZ) and Clotrimazole (CT).

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ods, the mobile phase generally comprises salt-containing reagents, such as potassium dihydrogenphosphate<sup>(11)</sup>. The HPLC assay for the determination of KCZ in common pharmaceutical preparations used acetonitrile phosphate buffer as the mobile phase<sup>(12)</sup>. In this study, a chromatographic method, where salt-containing reagents was not used in the mobile phase, was developed for the analysis of KCZ and CT in various drug matrices.

Because of the complexity of pharmaceutical matrix, a time-consuming sample pretreatment such as liquid-liquid extraction<sup>(16)</sup>, ultrasonic extraction<sup>(11-12)</sup> or acid degradation<sup>(13)</sup> is inevitably required. Supercritical fluid extraction (SFE) is a powerful sample preparation method for isolating pharmaceutical compounds, especially the relatively non-polar components from solid and semisolid pharmaceutical matrices<sup>(14)</sup>. CT and KCZ had been isolated from commercial pharmaceuticals using supercritical carbon dioxide at high pressure. For instance, Cavrini *et al.* isolated CT from commercial pharmaceutical preparations using 10% (v/v) methanol modified carbon dioxide at an extraction pressure of 380 bar (about 5510 psi)<sup>(16)</sup> where the cream sample was premixed with celite and anhydrous sodium sulfate. A new method that CT can be isolated from commercial pharmaceuticals at 2000 psi without premixing celite or the use of drying agents was approached in this study.

Here we described a sample preparation method based on SFE for the rapid isolation of KCZ and CT from various pharmaceutical formulations prior to the analysis by HPLC. The application for the assay of KCZ and CT in commercial pharmaceutical formulations was also investigated.

## MATERIALS AND METHODS

### I. Apparatus

SFE was performed using a supercritical fluid extractor (ISCO Model SFX 220, ISCO Inc., Lincoln, NE) equipped with a linear coaxially restrictor heater and a 50- $\mu\text{m}$  I.D. fused-silica capillary restrictor. ISCO 260D syringe pump was used to deliver  $\text{CO}_2$  to the extraction unit at constant pressure. All of SFEs were performed with 1 mL of acetonitrile as the modifier, which was directly added to a 10-mL extraction vessel with a total  $\text{CO}_2$  volume of 30 mL. The extraction flow rate of approximately 1.5 mL/min was maintained by a variable restrictor.

HPLC analysis was conducted using a Shimadzu LC 10AT equipped with a SIL-10AF auto injector, and an SPD-M10A diode array detector. A Waters  $\text{C}_{18}$  column (150 mm  $\times$  4.6 mm) and a Waters guard column (20 mm  $\times$  3.9 mm) were used. Ten microliter of sample was analyzed at a flow rate of 1.0 mL/min and monitored by the absorbance at 224 nm. Chromatographic data acquisition and processing were carried with a Class-VP 5.0 version interface controller.

### II. Reagents

Methanol and acetonitrile were of HPLC grade and purchased from Merck (Darmstadt, Germany). KCZ and CT were purchased from Sigma (St. Louis, MO, USA). Acetic acid and diethylamine were of analytical grade and also from Sigma. Deionized water was produced using a Millipore Milli-Q apparatus (Milford, MA, USA). Samples that contained KCZ and CT were purchased from several pharmaceutical stores in Taichung.

### III. Standard Solutions and Calibration

The stock solutions of KCZ or CT were prepared by dissolving 100 mg of KCZ or 100 mg of CT in the 100 mL of mobile phase, respectively. The working standard solutions of KCZ or CT were prepared by diluting the stock solution with the mobile phase in a 10-mL volumetric flask. The concentrations of KCZ working standard solutions were 25, 50, 100, 150 and 200  $\mu\text{g/mL}$ . The concentrations of CT working standard solutions were 50, 100, 150, 200 and 250  $\mu\text{g/mL}$ . These drug solutions were stable for at least two months at room temperature.

### IV. Sampling

Each pharmaceutical sample was weighed accurately (ca. 0.1 g) and placed in an empty 2.5-mL extraction vessel; it was spiked with 1 mL of acetonitrile, and the tablets were grounded using a mortar and pestle before SFE processing. After supercritical fluid extraction, the extract was collected in a 30-mL trap containing 10 mL of mobile phase solution at ambient temperature, followed by filtration through a 0.45- $\mu\text{m}$  Nylon-66 filter.

### V. Method Validation

The developed approach was verified by the precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The accuracy was determined by comparing the measured and label claim values of real pharmaceutical samples ( $n = 5$ ). The precision of the method was evaluated from the coefficient of variation (CV) of the five quantitative analyses. The LOD and LOQ, expressed as signal/noise = 3 and signal/noise = 10, were determined based on standard deviation through responses and the slope of regression equation of a curve constructed at lower concentration levels (15, 30, 45, 60 and 75  $\mu\text{g/mL}$  for CT, and 10, 20, 30, 40 and 50  $\mu\text{g/mL}$  for KCZ, respectively).

The accuracy of the proposed SFE was evaluated as the extraction recovery, which was defined as (found concentration/spiked concentration)  $\times$  100%. For extraction recoveries study, pharmaceutical samples spiked with 2, 4 and 6  $\mu\text{g}$  CT or KCZ standard solutions were extracted and analysed on the same day. The concentra-

tion responses were compared to those directly injected standards with the same amounts of CT or KCZ. The precision was evaluated by the coefficient of variation (CV) of triple extractions.

## RESULTS AND DISCUSSION

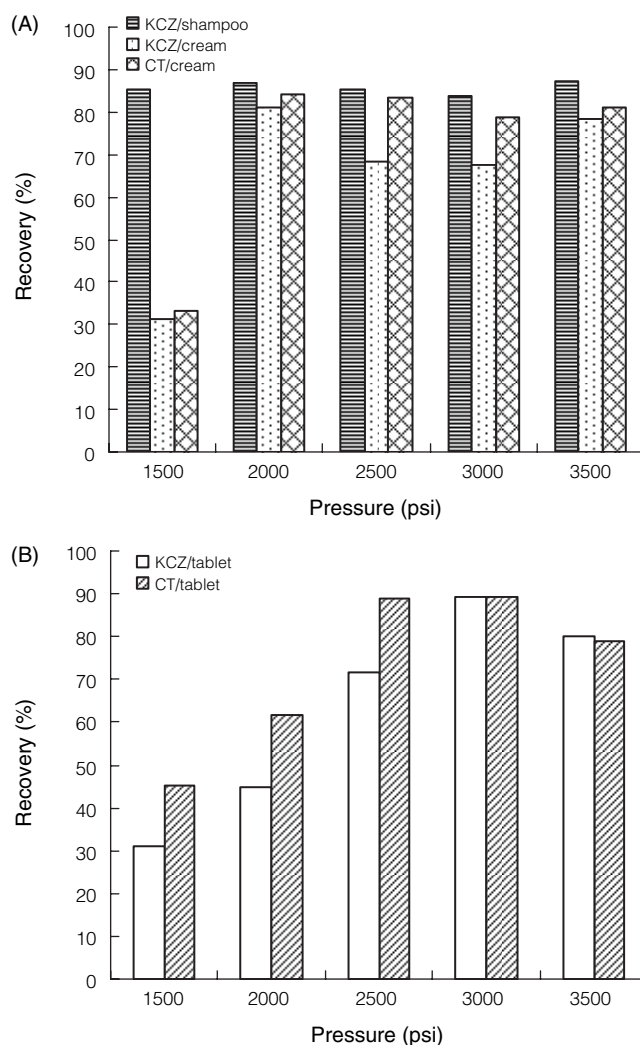
### I. Optimization of SFE Method

In this study, SFE conditions were optimized using a step-by-step design described by McNally<sup>(18)</sup>. A step-by-step approach is used as an alternative to the factorial design approach<sup>(19)</sup> due to similar results with less data processing. The effects of five parameters, namely extraction pressure, extraction temperature, the static extraction time, the dynamic extraction time and the amount of modifier added<sup>(20)</sup>, on the extraction efficiency of the SFE were investigated. Besides, there are three main extraction modes, namely static, dynamic and static-dynamic extractions in SFE. In static extraction, the penetration of the matrix by the fluid exceeds that in dynamic extraction, and the extraction time is usually less than in dynamic extraction mode. Static-dynamic extraction is more effective than static mode extraction and the consumption of supercritical fluid is lower. Therefore, samples are extracted in a static-dynamic mode with CO<sub>2</sub> throughout this study.

#### (I) Effect of Extraction Pressure

At first, extractions were performed at pressures from 1500 psi to 3500 psi in a 500 psi increment at fixed temperature (35°C) during a 10-min static extraction and a 10-min dynamic extraction periods using supercritical carbon dioxide, to investigate the effect of extraction pressures on the efficiency of SFE. The effects of pressure on the extraction recoveries of KCZ and CT from shampoo and cream matrices were presented in Figure 2 (A). Regardless of extraction pressure, the recoveries of KCZ from shampoo were in the range of 83.6% ~ 87.2%. However, increasing the extraction pressure from 1500 psi to 2000 psi markedly boosted the extraction recoveries for both KCZ cream and CT cream. Increasing the extraction pressure at fixed temperature increases the density of supercritical fluid and thus enhances the solvent strength of the supercritical fluid<sup>(18)</sup>. As the extraction pressure increased from 2000 psi to 3500 psi, the recoveries of the KCZ cream and CT cream were in the range 67.4% ~ 80.9%. Hence, subsequent extractions were performed at a pressure of 2000 psi for KCZ shampoo, KCZ cream and CT cream. It is also shown in Figure 2(A) that the recoveries of the KCZ cream and CT cream decreased as the extraction pressure increased from 2000 psi to 3500 psi. It was probably because the solubility of these analytes was considered less pressure dependent or poor collection efficiencies<sup>(20)</sup>.

In this study, the optimal extraction pressure for the SFE of KCZ cream and CT cream was 2000 psi, which was lower than that reported by Taylor *et al.*, which was around 300 bar (about 4350 psi) ~500 bar (about 7250 psi)<sup>(17)</sup>. Besides, CT was extracted from creams using SFE at 2000 psi, which was lower than that reported by Cavrini *et al.*<sup>(16)</sup>, 380 bar (about 5510 psi). With their SFE procedures, the time consumed for CT creams formulations was 4 min and a recovery of 100% was reported. In other studies, pharmaceuticals were extracted by SFE-CO<sub>2</sub> at 50°C at a pressure of 5800 psi for tablet formulations, and cream formulations were extracted by inverse SFE at 450 atm (about 6615 psi) and 60°C<sup>(17)</sup>. CT and KCZ were successfully isolated from various pharmaceuticals at extraction pressures lower than 3000 psi in this study. In previous researches, the extraction of tablets required higher pressure and temperature than the extraction of creams and shampoos<sup>(21)</sup>.



**Figure 2.** (A) Effect of pressures on the recoveries of KCZ and CT from shampoo and cream formulations by SFE. (B) Effect of pressures on the recoveries of KCZ and CT from tablets formulation by SFE.

The effect of pressure on the extraction efficiencies of KCZ tablets and CT tablets at constant temperature of 35°C during a 10-min static extraction and a 10-min dynamic extraction periods using supercritical carbon dioxide were evaluated herein. As shown in Figure 2(B), increasing of the extraction pressure from 1500 psi to 3000 psi considerably increased the recoveries for KCZ and CT tablets, indicating that increasing the pressure at a fixed temperature above the critical temperature (31.3°C) would improve the solvating power and the density of the supercritical carbon dioxide<sup>(18)</sup>. Since the extraction recovery slightly decreased at the pressure of 3500 psi, 3000 psi was taken as the optimal pressure for the extraction of KCZ and CT from tablet formulation. The extraction recoveries of KCZ and CT were 89.2% and 89.3%, respectively.

### (II) Effect of Extraction Temperature

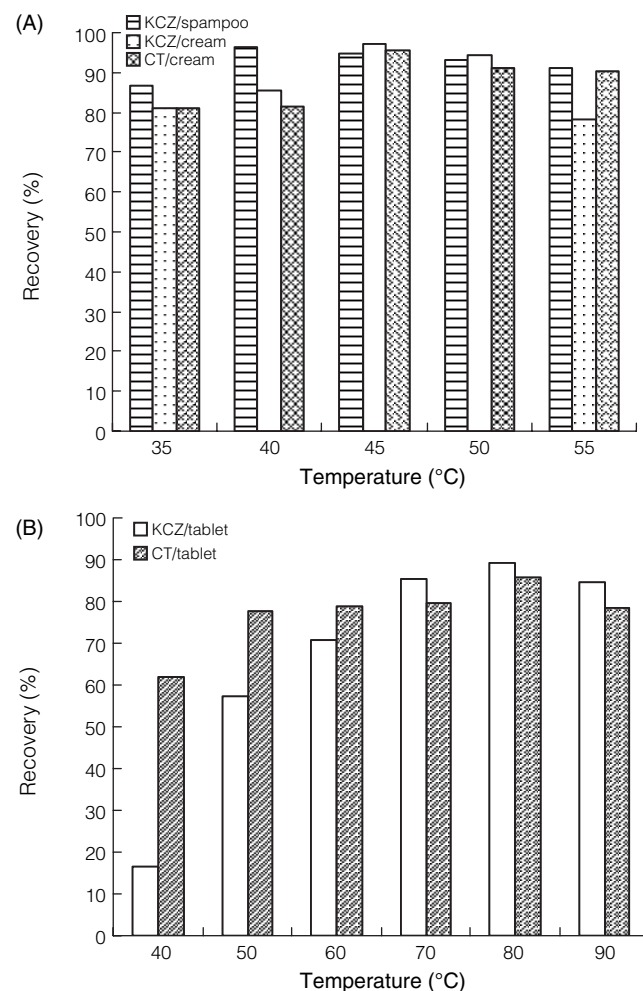
Increasing the temperature at a fixed pressure usually reduces the fluid density and the extraction efficiency, but in some cases, increases the extraction efficiency. The effect of temperatures on the extraction efficiencies depends on the analyte and the matrix. The effect of temperature (35, 40, 45, 50 and 55°C) at the optimized pressure (2000 psi) on the extraction efficiencies of KCZ shampoo, KCZ cream and CT cream during a 10-min static and a 10-min dynamic extraction periods using supercritical carbon dioxide was examined. Besides, the effect of temperature (40, 50, 60, 70, 80 and 90°C) on the extraction efficiencies of KCZ and CT tablets under the same condition were also investigated. As shown in Figure 3(A), increasing the extraction temperature from 35°C to 40°C in the extraction of KCZ from shampoo would improve recoveries from 86.8% to 96.4%. As the temperature was increased from 40°C to 55°C, similar extraction recoveries (94.8% ~ 91.2%) were observed. Higher temperature did not substantially improve the extraction recovery for KCZ shampoo. Supercritical fluid extraction of KCZ and CT from cream formulation at 45°C resulted in the highest recoveries (97.3% and 95.7%) in this work. Therefore, 45°C was chosen as the optimal extraction temperature for the SFE of KCZ and CT from both shampoo and cream formulations. As presented in Figure 3(B), increasing the extraction temperature of KCZ and CT from tablets dramatically increased the recovery at 3000 psi, which reached a maximum (89.2% and 85.8%) at 80°C. Accordingly, 80°C was chosen as the optimal extraction temperature for the SFE of KCZ and CT from tablet formulation.

### (III) Effect of Adding a Modifier

Although carbon dioxide is the most common supercritical fluid in SFE, pharmaceutical products containing active components are virtually insoluble in supercritical carbon dioxide fluid. Addition of a small amount of

modifier (generally organic solvents) increases the solvent strength of the fluid or improves the desorption of analytes from the sample matrices, thus improving the extractability of target analytes<sup>(22)</sup>. There are two modes of modifier addition, dynamic mode or static mode. On the other hand, the modifier can be directly added into the sample matrix in static mode. Previous researches about the extraction of pharmaceutical formulations indicated that the addition of the modifier to the sample matrix prior to the extraction would improve the extraction efficiency<sup>(23)</sup>.

Acetonitrile (ACN) was the best static modifier used to spike the organic solvent tested herein. The modifier was added directly into the 10-mL extraction vessel, with the range of 1% ~ 10% (v/v) as described by Yamini *et al.*<sup>(20)</sup>. The percentage of ACN was determined by the volume of ACN divided by the extraction vessel volume (10 mL) in static mode. The modifier was added directly into the extraction vessel, and swept from the extraction vessel, where the supercritical fluid started to circulate through the sample. Therefore, the modifier would increase the



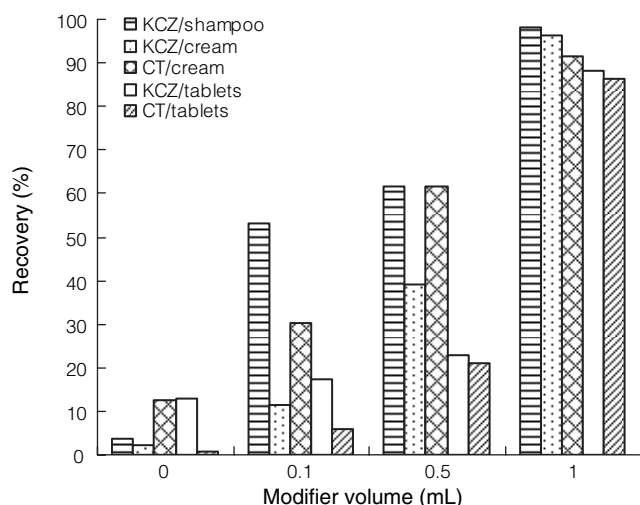
**Figure 3.** (A) Effect of temperatures on the recoveries of KCZ and CT from shampoo and cream formulations by SFE. (B) Effect of temperatures on the recoveries of KCZ and CT from tablets formulation by SFE.

solubility of samples in the supercritical fluid<sup>(24)</sup>.

The effects of the volume of ACN, 0 (0%), 0.1 mL (0.1%), 0.5 mL (5%) and 1 mL (10%), on the extraction recovery were demonstrated in Figure 4. For KCZ formulations, when 1 mL of ACN was added in the vessel, the extraction recoveries were 30% ~ 66% higher than that obtained by pre-spiking with 0.5 mL of ACN; the same result was obtained for the CT formulations. Hence, SFE with 1 mL-acetonitrile-modified carbon dioxide was applied throughout this study. Pre-spiking with 1 mL of acetonitrile improved the extraction efficiency of the fluid by a “co-extraction effect”.

#### (IV) Effect of Extraction Time

In previous experiments, the extraction time was fixed at 10 min for both static and dynamic extractions with recoveries lower than 90.0 %. In order to obtain better recovery, three static time (10, 15, 20 min) followed by 15 min of dynamic extraction tested at the optimized pressure and temperature. With 10-min static extraction, the recoveries of KCZ from shampoo, cream and tablets were 85.2%, 86.3% and 82.4%, respectively. The recoveries of CT from cream and tablets were 87.2% and 86.9%, respectively. No significant improvement in the extraction recoveries of KCZ and CT was observed by longer static time (15 min or 20 min). The penetration of the matrix by the fluid in static extraction exceeds that in dynamic extraction, and the effect of static time on the extraction efficiency depends on the analyte and the matrix. Therefore, 10 min was chosen as the optimal static extraction time for extracting KCZ and CT from various matrices. On the other side, three dynamic times (10, 15, 20 min) following 10 min of static extraction at the optimal pressure and temperature were examined.



**Figure 4.** Effect of modifier volume on extraction efficiency of KCZ and CT from various pharmaceuticals. (SFE was performed with 2000 psi at 45°C for creams and for shampoo and with 3000 psi at 80°C for tablets, respectively.)

By 10 min-dynamic extraction, the recoveries of KCZ from shampoo, cream and tablets were 85.2%, 86.3% and 82.4%, and CT from cream and tablets, 87.2% and 86.9%, respectively. Increasing the dynamic extraction time to 15 min did improve the recoveries of KCZ and CT from shampoo, cream and tablets, ranging from 92.6% to 101.5%. However, increasing the dynamic extraction time to 20 min did not improve the recoveries. Therefore, 15 min was chosen as the optimal time for the dynamic extraction of KCZ and CT from various matrices.

#### II. Validation of the Developed Method

The pH value of the mobile phase was adjusted between 4.0 and 6.0 in most liquid chromatographic methods for analyzing KCZ<sup>(25-26)</sup>. KCZ undergoes hydrolysis in alkaline solution with a loss of peak symmetry, while it is stable in ethanolic solution at room temperature<sup>(27)</sup>. On the other side, CT is stable in alkaline medium but hydrolyzes into imidazole and (2-chlorophenyl)-diphenyl methanol in acidic medium<sup>(17,28)</sup>. The optimal pH value of the mobile phase for the analysis of both CT and KCZ tested is 7.0 in this work. The mobile phase solution with a pH value more or less than 7.0 resulted in poor precision. Therefore, for safe consideration, the appropriate pH of the mobile phase for isolating CT and KCZ from admixtures was finally adjusted to 7.0 herein.

An important merit of this proposed method compared to other chromatographic methods is that the mobile phase was prepared without salt-containing reagents. It substantially reduced the possibility of crystallization and column cleaning time and extended the life of the column. The mobile phase used to separate KCZ and CT consisted of methanol/water/diethylamine/glacial acetic acid (80:20:0.3:0.2, v/v) in this study. The addition of diethylamine or equivalents to the mobile phase as a masking reagent prevented the tailing of peaks and increased the retention time by the interaction between basic drugs and silica gels<sup>(29)</sup>.

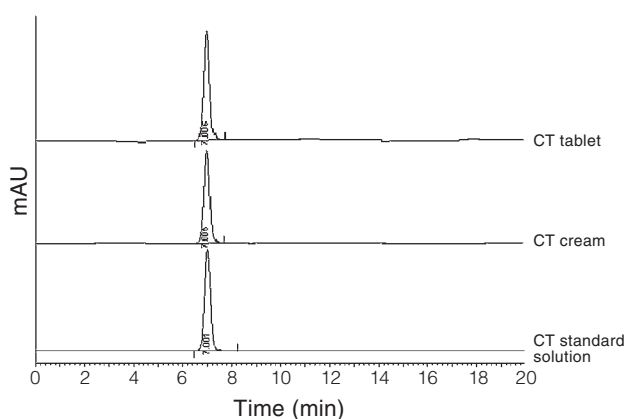
The calibration curves of KCZ and CT were obtained by regression analyses of peak areas against concentrations. The calibration curve was plotted over the range of 25 ~ 250 µg/mL for KCZ with a correlation coefficient of 0.9994. The calibration curve for CT was over the range of 50 ~ 250 µg/mL with a correlation coefficient of 0.9998. In this method, the retention times of CT standard and CT from various matrices were nearly the same (7.01 min, 7.01 min and 7.01 min) as shown in Figure 5. The retention times of KCZ standard and KCZ from various matrices were 4.96 min, 4.98 min, 5.00 min and 4.96 min, as shown in Figure 6.

The proposed methods were applied to the determination of KCZ and CT in standard and commercial pharmaceutical samples. The accuracy of the proposed SFE approach was evaluated as the extraction recovery, which was defined as (found concentration/spiked concentration) × 100%, and satisfactory recoveries ranging from

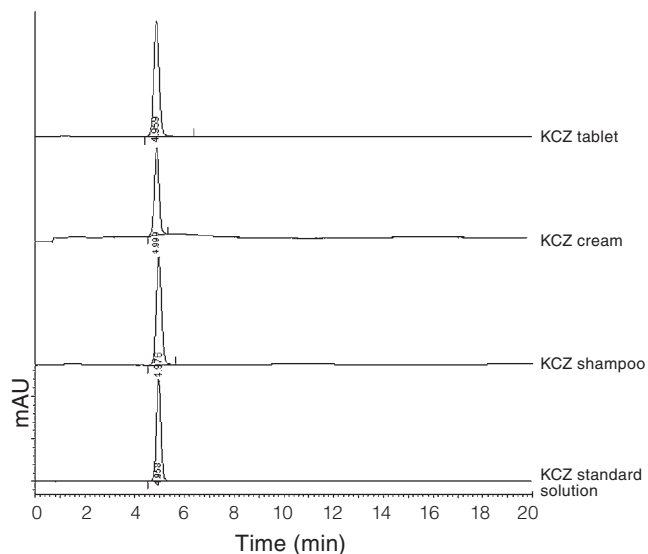
90.6% to 100.1% were obtained as shown in Table 1. The precision was evaluated by the coefficient of variation (CV) of triple extractions. The developed SFE approach for the analysis of CT and KCZ was validated as accurate and precise with low CV ranging from 1.04% to 5.93%.

The accuracy and precision of this proposed methods were also evaluated by the quantitative analysis CT or KCZ from different samples (n = 5) as shown in Table 2. KCZ was extracted from different formulations under the optimal SFE conditions. It resulted in satisfactory extraction efficiencies of 92.2% ~ 97.1% and CV values of less than 2.80%. The LOD and LOQ for KCZ were determined to be 2.31 and 7.70 µg/mL, respectively,

which were lower than that of HPLC method by Velikina *et al.*<sup>(14)</sup>, for which the corresponding values were 2.52 µg/mL and 6.25 µg/mL, respectively. The extraction of CT from creams and tablets under the optimal SFE conditions resulted in satisfactory recoveries ranging from 90.8% to 97.2% from five replicate determinations and CV values of less than 3.98%. The LOD and LOQ of CT were determined to be 0.42 µg/mL and 1.40 µg/mL, respectively in this study, and the LOD was



**Figure 5.** HPLC chromatograms of CT in various pharmaceutical formulations. Column: C<sub>18</sub> column (150 mm × 4.6 mm); detection: 224 nm; eluent: MeOH/H<sub>2</sub>O/diethylamine/glacial acetic acid (80:20:0.3:0.2, v/v); flow rate: 1.0 mL/min.



**Figure 6.** HPLC chromatograms of KCZ in various pharmaceutical formulations. Column: C<sub>18</sub> column (150 mm × 4.6 mm); detection: 224 nm; eluent: MeOH/H<sub>2</sub>O/diethylamine/glacial acetic acid (80:20:0.3:0.2, v/v); flow rate: 1.0 mL/min.

**Table 1.** Recoveries of spiked standard solutions to pharmaceutical samples<sup>a</sup>

	Spiked mass (µg)	Mass detected ± SD (µg) <sup>b</sup>	Recovery (%) ± SD <sup>b</sup>	CV (%) <sup>b</sup>
Cream/KCZ	2.00	1.82 ± 0.06	90.59 ± 3.27	3.61
	4.00	3.68 ± 0.05	91.76 ± 1.39	1.52
	6.00	5.81 ± 0.08	96.25 ± 1.61	1.67
Shampoo/KCZ	2.00	1.92 ± 0.03	95.37 ± 1.97	2.07
	4.00	4.03 ± 0.04	100.07 ± 1.17	1.17
	6.00	5.78 ± 0.19	95.83 ± 3.47	3.62
Tablet/KCZ	2.00	1.91 ± 0.04	95.25 ± 2.02	2.12
	4.00	3.76 ± 0.07	93.92 ± 1.74	1.85
	6.00	5.85 ± 0.06	97.55 ± 1.01	1.04
Cream/CT	2.00	1.89 ± 0.08	91.57 ± 5.43	5.93
	4.00	3.64 ± 0.04	90.96 ± 1.34	1.48
	6.00	5.63 ± 0.18	93.75 ± 3.38	4.09
Tablet/CT	2.00	1.93 ± 0.03	96.42 ± 1.52	1.58
	4.00	3.84 ± 0.11	95.98 ± 2.94	3.06
	6.00	5.77 ± 0.14	96.14 ± 2.29	2.39

<sup>a</sup>SFE was performed with 10% ACN modifier. SFE was performed with 2000 psi at 45°C for creams and for shampoos and with 3000 psi at 80°C for tablets, respectively.

<sup>b</sup>Each value is the mean of triple extractions. The recovery was confirmed by HPLC-DAD.

**Table 2.** Quantitative results of KCZ and CT in commercial pharmaceuticals<sup>a</sup>

Sample	Labeled ingredient /amount	Quantitative results	Extraction efficiency (%) <sup>b</sup>	CV (%) <sup>b</sup>
Shampoo 1	1% KCZ (w/w)	0.97 ± 0.01% KCZ (w/w)	97.20 ± 1.14	1.17
Shampoo 2	2% KCZ (w/w)	1.94 ± 0.00% KCZ (w/w)	97.09 ± 0.03	0.03
Shampoo 3	2% KCZ (w/w)	1.90 ± 0.00% KCZ (w/w)	95.12 ± 0.01	0.01
Cream 1	20 mg KCZ/g cream	19.13 ± 0.11 mg KCZ/g cream	95.65 ± 0.54	0.56
Cream 2	20 mg KCZ/g cream	18.93 ± 0.25 mg KCZ/g cream	94.63 ± 1.26	1.33
Cream 3	10 mg CT/g cream	9.13 ± 0.13 mg CT/g cream	90.75 ± 1.10	1.21
Cream 4	10 mg CT/g cream	9.72 ± 0.37 mg CT/g cream	97.01 ± 3.86	3.98
Tablet 5	20 mg KCZ/g tablet	18.44 ± 0.52 mg KCZ/g tablet	92.20 ± 2.58	2.80
Tablet 6	6.67 mg CT/g table	6.05 ± 0.12 mg CT/g tablet	90.77 ± 1.87	2.06

<sup>a</sup>SFE condition was the same as Table 1. The quantitative results were confirmed by HPLC-DAD.

<sup>b</sup>Each value is the mean of five extractions, mean ± SD.

lower than that of HPLC method by Lin and Wu<sup>(15)</sup>, for which the corresponding value was 0.5 µg/mL.

### CONCLUSIONS

The proposed method used for CT and KCZ analysis in shampoos, creams and tablets was precise and reproducible, which provided a valuable method for the quality control of these two drugs in the studied matrices. The effects of pressure, temperature, extraction time and modifier concentration on the extraction efficiency of CT and KCZ from various pharmaceutical matrices were investigated. With the proposed HPLC method with SFE, it is possible to detect CT and KCZ down to 0.42 µg/mL and 2.31 µg/mL, respectively. Besides, a good agreement was obtained between experimental values and labeled values.

### ACKNOWLEDGEMENTS

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