Abstract. The aim of the present study was to investigate the characteristics of Su Xiao Jiu Xin dripping pill absorption in the buccal mucosa of healthy volunteers. This pill is a traditional Chinese medicine that is widely used as an emergency treatment for cardiovascular and cerebrovascular diseases. It is sublingually administered and can be absorbed in the buccal mucosa. In the present study, a method was developed to investigate the absorption characteristics in the buccal mucosa of healthy volunteers via a circulating device by gas chromatography-mass spectrometry and high-performance liquid chromatography. The five main efficacy components associated with cardiovascular and cerebrovascular diseases, which are borneol, isoborneol, ligustilide, n-butylphthalide and ferulic acid, were detected and rapidly absorbed. Among these components, four exhibited good absorption, thus confirming that the method developed is efficient for analysis of the absorption characteristics.

Introduction

Su Xiao Jiu Xin dripping pill (SXJXDP) is a traditional Chinese medicine (TCM), which is widely used to treat cardiovascular and cerebrovascular diseases. SXJXDP is composed of Chuanxiong (Rhizoma Chuanxiong) and Bing pian (Borneolum) (1). Phytochemical studies have shown that SXJXDP contains numerous types of compounds, such as borneol and isoborneol from Borneolum, lactones (ligustilide and n-butylphthalide) and organic acids (ferulic acid) from Rhizoma Chuanxiong (2,3). Pharmacological studies have also revealed that major active components, including borneol, isoborneol, ligustilide, n-butylphthalide and ferulic acid, are implicated in the treatment of cardiovascular and cerebrovascular diseases (4-7). Therefore, an efficient method should be developed to detect these compounds absorbed in the buccal mucosa.

SXJXDP is sublingually administered and can be absorbed by the rich blood flow in the buccal mucosa. As a non-keratinising epithelial tissue, the buccal mucosa provides a number of advantages in drug permeability, prevents first-pass metabolism in the liver exhibiting good compliance depending on a particular drug and contains efficient enzymatic flora (8,9). Therefore, the characteristic of drug absorption in the human buccal mucosa should be determined to provide relevant guidelines. However, to the best of our knowledge, the release pattern and absorption characteristics of the main bioactive components of SXJXDP in the human buccal mucosa have not yet been reported.

In the present study, borneol, isoborneol, n-butylphthalide and ligustilide were detected by gas chromatography-mass spectrometry (GC-MS). Ferulic acid was analysed by high-performance liquid chromatography (HPLC). The circulating system involved in the drug absorption in the buccal mucosa was observed to investigate the absorption characteristics of the five main bioactive components, which are associated with cardiovascular and cerebrovascular diseases. Furthermore, an absorption curve was illustrated and permeability coefficient was calculated. The study aimed to establish efficient methods and explain the absorption characteristics of SXJXDP.

Materials and methods

Materials. The standard compounds of borneol, isoborneol, ligustilide, n-butylphthalide and ferulic acid were purchased from the National Institutes for Food and Drug Control (Beijing, China). HPLC-grade acetonitrile, n-hexane and dichloromethane were purchased from Oceanpak Axelative Chemical (Gothenburg, Sweden). Pure water was purchased...
from Wahaha Group Co. (Hangzhou, China). SXJXDP was purchased from the No. 6 Pharmaceutical Factory of Tianjin Zhongxin Pharmaceutical Co. (Tianjin, China). Six healthy volunteers underwent training and signed an informed consent prior to the experiment. Our study was approved by the ethics committee of Tianjin University of Traditional Chinese Medicine. These volunteers gargled pure water prior to the experiment. The presence or absence of adverse reactions was closely observed during the process and whether the mucosa remains normal or becomes abnormal following the completion of the experiment was assessed.

In total, 15 pills were dissolved in 50 ml purified water and added to the circulating fluid storage cup of the circulating devices. The buccal mucosa devices used in the experiment were prepared in accordance with previously described methods (10,11). Fig. 1 illustrates the diffusion cell used in the study. This diffusion cell is composed of polytetrafluoroethylene and comprises two sides; a-side functions as an inlet of circulating fluid and b-side serves as an outlet of circulating fluid (10,11). The circulating system was equipped with a magnetic stirrer and performed under the following conditions: A suitable modulation speed and solution temperature of 37°C. At 0, 1, 3, 5, 10, 20, 30, 45, 60 and 90 min, 300 µl of the solution was obtained. Subsequent to performing the sampling, the same volume of blank solution was immediately injected as a supplementary sample.

**Chromatography.** Borneol, isoborneol, ligustilide and n-butyphthalide were analysed using a GC-MS-QP-2010 SE (Shimadzu, Japan) with a column RtX-5MS type at an inlet temperature of 280°C, an ion source temperature of 200°C, a flow rate of 1 ml/min helium carrier gas and an injection volume of 1 µl in a splitless mode.

Borneol and isoborneol samples were prepared under the following chromatographic conditions. In brief, 50 µl sample was obtained and 300 µl n-hexane were added. Subsequently, dichloromethane (9:1 v/v) liquid-to-liquid extraction was performed by vortexing for 1 min, and the vortexed extract was centrifuged at 9,168 x g for 5 min. An aliquot of the supernatant was subsequently obtained. The temperature program started at 70°C for 2 min and was increased at a rate of 5°C/min until 120°C was reached. Temperature was ramped at 50°C/min until a final temperature of 280°C was achieved. The temperature was maintained constantly at 280°C for 2 min.

Ligustilide and n-butyphthalide samples were prepared under the following chromatographic conditions. In brief, 100 µl sample was obtained and 100 µl n-hexane was added. Subsequently, dichloromethane (9:1 v/v) liquid-to-liquid extraction was performed by vortexing for 1 min. The vortexed extract was centrifuged at 9,168 x g for 5 min. An aliquot of the supernatant was subsequently obtained. The temperature program started at 70°C for 2 min and was increased at a rate of 5°C/min until 120°C was reached. Temperature was ramped at 50°C/min until a final temperature of 300°C was reached, and was subsequently maintained constantly at 300°C for 2 min.

Ferulic acid sample was prepared under the following chromatographic conditions. In brief, 100 µl sample was filtered using a 0.45 µm membrane and injected into HPLC-LC-20AT (Shimadzu, Japan). HPLC was performed in a Diamonsil C18 column (4.6x250 mm, 5 µm) with a mobile phase of methanol: 1% acetic acid aqueous solution (42:58 v/v) at a flow rate of 1 ml/min. The column temperature was maintained at 25°C and the injection volume was 20 µl. The wavelength of the detector was set at 321 nm.

The cumulative absorption rate was calculated to evaluate drug absorption and was obtained using the following equation: \( \frac{(F_t - F_0)/F_0 \times 100\%}{t} \), where \( F_0 \) is the liquid concentration at time \( t \) and \( F_t \) is the initial concentration of the solution.

The permeability coefficient was calculated to evaluate drug permeability. Permeability coefficient was obtained using the following equations: \( J = \frac{dQ}{dt} = \frac{P \times (C_s - C_d)}{C_s} = \frac{P \times C_s}{C_d} \), where \( Q \) is the drug cumulative infiltration capacity in µg/cm²; \( t \) is the time in min; \( P \) is the permeability coefficient in cm²/s; and \( C_s \) and \( C_d \) are the drug concentrations of the supply cell and the accepting cell in µg/ml, respectively. Additionally, \( C_s >> C_d \).

Under these conditions, three sensitive and reliable analytical methods were developed to simultaneously quantify borneol, isoborneol, ligustilide and n-butyphthalide by GC-MS and analyse ferulic acid by HPLC. The representative chromatograms of the compounds indicated that the developed method was free of any evident interference.

**Results and Discussion**

GC-MS and HPLC methods were validated in terms of linearity, precision, accuracy, stability and recovery. Calibration curves were constructed at five different concentration levels from the following concentration ranges: 2-200 µg/ml for borneol; 2-400 µg/ml for isoborneol; 1-100 µl/ml for ligustilide; 0.1-15 µl/ml for n-butyphthalide; and 1.081-16.215 µg/ml for ferulic acid. Each calibration curve was obtained from five different concentrations of the five bioactive analytes by linear regression analysis. The obtained correlation coefficient values (\( r^2 >0.999 \)) indicated good correlations between the concentrations and their peak responses within the test ranges. Stability test results showed that the processed samples could be re-injected after 0, 2, 4, 8 and 12 h; furthermore, the relative standard deviation (RSD)% of the stabilities of these five bioactive analytes ranged, 1.11-8.71%, which was within acceptable ranges. The precision of these methods was determined by...
Repeated injections (n=6) of the standard solutions; the accuracy of these methods was determined by injections (n=6) of equally treated samples. The RSD% values of the precision and the accuracy of five bioactive analytes ranged, 1.12‑5.01% and 1.85‑8.71%, respectively. The recoveries of the GC‑MS analytes were compared to a standard solution following extraction using known concentrations at three different levels. The recoveries of the HPLC analytes were determined by spiking the samples with known amounts of the standard solution at three different concentration levels. The recoveries of the five analytes were as follows: 88.96‑91.63% for borneol; 86.27‑87.84% for isoborneol; 84.67‑89.25% for ligustilide; 84.72‑88.99% for n‑butylphthalide; and 100.07 -103.77% for ferulic acid. Overall, the recoveries of each of the analytes at three different concentrations were consistent and >80%. These results demonstrated that all the values were present in permissible ranges and the methods were accurate (Table I).

The buccal mucosa absorption curve showed that the five types of components reached a plateau in 10‑20 min. During this period, the maximum cumulative absorption rate was almost obtained. Subsequently, the cumulative absorption rate was significantly decreased. The cumulative absorption rates of borneol, isoborneol, ligustilide and n‑butylphthalide showed that the maximum accumulation in 60 min were 86.70, 93.16, 94.25 and 93.16%, respectively. The accumulation of the four components was almost complete. In the buccal mucosa absorption curve of ferulic acid in 60 min, the maximum absorption rate was 15.91% with incomplete absorption and each component exhibited different performances (Fig. 2).

Ferulic acid yielded the lowest permeability coefficient; by comparison, the four other components exhibited relatively similar permeability coefficients. However, the penetration ability of ferulic acid in the buccal mucosa was ~10 times weaker than that of the four other components (Table II).

Previous studies showed that SXJXDPs are stable during absorption (1). This phenomenon can account for the decrease in drug concentration in vitro, indicating that this decrease is not caused by drug degradation; instead, this decrease occurs as a result of drug absorption into the bloodstream. Therefore, residual drug method was applied in the present study to evaluate absorption.

Components undergo rapid sublingual absorption as the buccal mucosa is extremely thin and rich in blood vessels. These components can enter blood circulation within an extremely short time to alleviate symptoms and provide first‑aid relief. Drug absorption occurs in two passages, via intracellular and intercellular routes, which primarily exhibit passive diffusion influenced by several physicochemical properties, such as lipid solubility, dissociation degree, molecular weight and permeability effect.

In general, drugs with low molecular weights are easily absorbed by mucosa capillaries. The five main bioactive components exhibit molecular weights <200 Da; as such, these components can pass through the buccal mucosa membrane and into the bloodstream. As a drug‑promoting agent in TCM, borneol exhibits strong absorption and permeation abilities, and can also promote the absorption of other substances in the buccal mucosa to a certain extent (12). Lipophilic drugs can easily penetrate intercellular channels filled with lipid. This penetration is possible as sublingually

**Table I. Result of the method validation.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Regression equation</th>
<th>r</th>
<th>Precision, RSD%</th>
<th>Accuracy, RSD%</th>
<th>Stability, RSD%</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borneol</td>
<td>y=1.311x10^5x-3.329x10^4</td>
<td>0.9998</td>
<td>3.58</td>
<td>2.44</td>
<td>2.63</td>
<td>88.96</td>
</tr>
<tr>
<td>Isoborneol</td>
<td>y=4.148x10^5x+3.203x10^5</td>
<td>0.9994</td>
<td>4.54</td>
<td>2.95</td>
<td>2.46</td>
<td>86.80</td>
</tr>
<tr>
<td>Ligustilide</td>
<td>y=2.594x10^4x-2.317x10^4</td>
<td>0.9998</td>
<td>5.01</td>
<td>4.86</td>
<td>8.71</td>
<td>88.77</td>
</tr>
<tr>
<td>n‑butylphthalide</td>
<td>y=1.631x10^3x-8.477x10^3</td>
<td>0.9996</td>
<td>2.73</td>
<td>8.71</td>
<td>3.86</td>
<td>87.50</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>y=1.112x10^3x-3.830x10^3</td>
<td>0.9998</td>
<td>1.12</td>
<td>1.85</td>
<td>1.11</td>
<td>103.77</td>
</tr>
</tbody>
</table>

RSD, relative standard deviation.

**Table II. Permeability coefficient of buccal mucosa.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>P, cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borneol</td>
<td>1.12x10^-2</td>
</tr>
<tr>
<td>Isoborneol</td>
<td>1.17x10^-2</td>
</tr>
<tr>
<td>Ligustilide</td>
<td>1.45x10^-2</td>
</tr>
<tr>
<td>n‑butylphthalide</td>
<td>1.34x10^-2</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>1.93x10^-3</td>
</tr>
</tbody>
</table>

Figure 2. Time course of the cumulative absorption rates of the five components of Su Xiao Jiu Xin dripping pill. Values are mean ± standard deviation.
administered drugs permeability coefficients closely associated with the oil-water partition. \( K \). \( K \) is extremely high when fat-soluble compounds are insoluble in saliva. By contrast, \( K \) is extremely low when compounds are strongly hydrophilic and membrane permeability is weak. The Ideal \( K \) ranges, 40-2,000 (13,14). Borneol, isoborneol, \( n \)-butylphthalide and ligustilide are fat-soluble compounds; therefore, these compounds are absorbed more efficiently and rapidly in the buccal mucosa compared to ferulic acid. In contrast to the four fat-soluble compounds, ferulic acid exhibits strong hydrophilicity, poor permeation ability, poor absorption and weak permeability in the buccal mucosa. In addition, the pKa values of ferulic acid are 4.56 and 8.65; the main form of ferulic acid in saliva (pH=6.6-7.1) (15) is a mono-anion (16). Drugs are transported across the cell membrane in an absorbable molecular state. However, the present form of ferulic acid is one of the key reasons of its low absorption. Therefore, drug absorption in the buccal mucosa is an extremely complicated process.

In conclusion, the GC-MS and HPLC methods were established in the present study to detect the bioactive components of SXJXDP. These methods may be suitably applied to elucidate the characteristics and permeabilities of drugs in the buccal mucosa of healthy volunteers, as validated by comprehensive quality control strategies of drugs absorbed in the buccal mucosa.

**Acknowledgements**

The present study was financially supported by the Large Variety of Technological Innovation of Su Xiao Jiu Xin Dripping Pill (grant nos. 2011ZX09201-201 and 2011ZX09201-201-2).

**References**