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# Development of a novel combination tablet containing trimebutine maleate and mosapride citrate for the treatment of functional dyspepsia

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ABSTRACT

To develop a novel combination tablet which contained 100 mg trimebutine maleate and 5 mg mosapride citrate (TMCT) for the treatment of functional dyspepsia, the wet granulation method was used to prepare TMCTs with various amounts of diluents and stabilizers. The levels of impurities, the stability and the dissolution of the TMCTs were investigated. The oral bioavailability of drugs in the TMCTs was then evaluated and compared to the simultaneous oral administration of trimebutine maleate-loaded and mosapride citrate-loaded commercial products in the beagle dog. Among the diluents tested, D-mannitol was selected, since the microcrystalline cellulose and lactose did not inhibit the production of drug impurities due to their hygroscopic properties and chemical interactions, respectively. Furthermore, succinic acid was selected as the stabilizer because it gave the lowest level of total drug impurities of the organic acids tested. The combination tablet of trimebutine maleate and mosapride citrate prepared with D-mannitol and succinic acid gave a total drug content higher than 95% and total impurities lower than 0.5% at 25 °C/60% RH and 40 °C/75% RH during a 6-month period, indicating that the tablets were stable for at least 6 months. Furthermore, this combination tablet showed a similar dissolution to the trimebutine maleate-loaded and mosapride citrate-loaded commercial products and gave insignificantly different absorption compared to these commercial products in beagle dogs. Thus, the combination tablet of trimebutine maleate and mosapride citrate prepared with D-mannitol and succinic acid would be a stable and effective oral pharmaceutical product for the treatment of functional dyspepsia.


## 1. Introduction

Functional dyspepsia is a clinical syndrome characterized by persistent or recurrent pain or discomfort in the upper gastrointestinal tract without evidence of organic disease (Talley et al., 1999). These complex symptoms, which are often worsened by food, include epigastric pain, bloating, early satiety, fullness, epigastric burning, belching, nausea and vomiting. Surveys indicated that 15–20% of the general population suffered from functional dyspepsia over the course of 1 year (Agreus et al., 1994; Drossman et al., 1993; Talley et al., 1992). Furthermore, 20–30% of functional dyspepsia patients were found to suffer from comorbidities of irritable bowel syndrome or other gastrointestinal disorders (Talley and Piper, 1985).

A combination therapy containing mosapride citrate [(*RS*)-4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)morpholin-2-yl]methyl}benzamide citrate] (Fig. 1) and trimebutine maleate [3,4,5-Trimethoxybenzoic acid 2-(dimethylamino)-2-phenylbutyl ester maleate] (Fig. 1) was selected for the effective treatment of complex symptoms related to functional dyspepsia (Talley, 1992). Mosapride, a selective 5-HT5 agonist, is known to noticeably promote upper gastrointestinal motility, such as gastric emptying, with no severe side effects, such as prolongation of interval between Q and T wave in the electrocardiogram (Ruth et al., 1998). Trimebutine is classified as the only enkephalin receptor agonist that is spread throughout the entire gastrointestinal tract. This drug can regulate the hypo- and hyper-motility of the broad gastrointestinal tract with a dual action depending on the enkephaline receptor subtype (Taniyama et al., 1991). Trimebutine is also effective for the treatment of irritable bowel syndrome or strange colon pain (Lüttecke, 1980). For efficient control of the complex symptoms of functional dyspepsia, an effective dosage form of trimebutine maleate and mosapride citrate needs to be developed.

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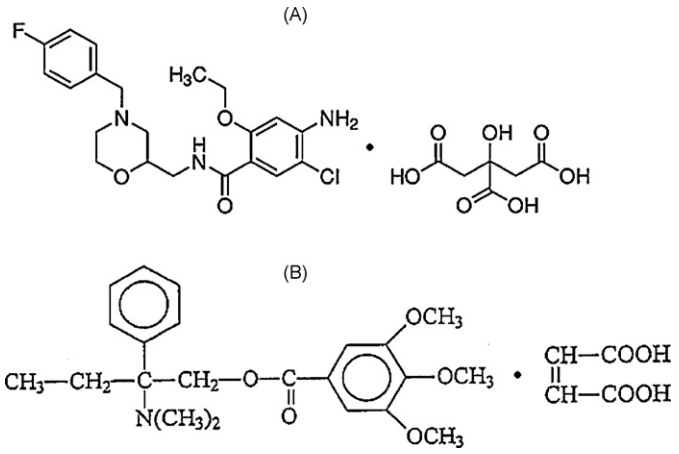


**Fig. 1.** Chemical structure: (A) mosapride citrate and (B) trimebutine maleate.

In this study, in order to develop a novel combination tablet containing trimebutine maleate and mosapride citrate (TMCT), the wet granulation method was used to prepare TMCTs with various amounts of diluents and stabilizers. The impurities, stability and dissolution of the TMCTs were investigated and the oral bioavailability of the drugs was then evaluated compared to the simultaneous oral administration of trimebutine maleate-loaded (Polybutine®) and mosapride citrate-loaded commercial products (Gasmotin®) in the beagle dog. In this study, organic acids such as citric acid, tartaric acid and succinic acid were used as stabilizers. It was reported that the hydrolysis of trimebutine in aqueous solution was considerably inhibited by organic acids (Park and Rhee, 1990). Moreover, the hydrolysis of ester compounds was reduced by the micro-environmental acidic pH in the solid state (Badaway et al., 1999; Thumma et al., 2008).

## 2. Materials and methods

### 2.1. Materials

Trimebutine maleate was purchased from ZaCh Systems s.p.a. (Milano, Italy). Mosapride citrate was purchased from Dongwoo Syntech (Geyung-gi, South Korea). D-Mannitol, microcrystalline cellulose, silicon dioxide, croscarmellose sodium, lactose anhydrous, lactose monohydrate, hydroxypropyl cellulose, magnesium stearate, citric acid, tartaric acid and succinic acid were provided by Samil Pharm. Co. (Anyang, South Korea) and were of USP grade. The trimebutine maleate-loaded commercial product (Polybutine®; in tablet form) was supplied by Samil Pharm. Co. (Anyang, South Korea). The mosapride citrate-loaded commercial product (Gasmotin®; in tablet form) was purchased from Daewoong Pharm. Co. (Seungnam, South Korea). All other chemicals were of reagent grade and were used without further purification.

### 2.2. Animals

All animal care practices and experimental procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989, revised in 1999 and amended in 2008 by the Society of Toxicology (SOT, 2008). The protocols for the animal studies were also approved by the Institute of Laboratory Animal Resources of Yeungnam University. Eight male beagle dogs weighing 12–13 kg were fasted for 16 h prior to the experiments with free access to water.

### 2.3. Impurities of drugs/degradation study

For the standard solutions, 100 mg trimebutine maleate or 5 mg mosapride citrate was dissolved in a mixture of 0.01N HCl and acetonitrile (65:35, v/v) (1 l). The trimebutine maleate-loaded tablet or a mosapride citrate-loaded tablet at the equivalent dose of 100 mg/l trimebutine maleate or 5 mg/l mosapride citrate was added to a mixture of 0.01N HCl and acetonitrile (65:35, v/v) (1 l). The trimebutine maleate-loaded tablet and the mosapride citrate-loaded tablet were prepared with commonly used ingredients such as microcrystalline cellulose and magnesium stearate using direct compression. These standard solutions and samples were vortexed for 30 min and filtered through a membrane filter (0.45 μm) to obtain a clear solution. To assay their drug contents and impurities, the resulting solutions (20 μl) were analysed by HPLC (Agilent 1200, Wilmington, DE, USA) equipped with a C18 column (Symmetry®; Waters, 5 μm, 150 mm × 3.9 mm i.d.) and a UV detector (Model L-7450, Agilent Technologies Inc., Wilmington, DE, USA). The mobile phase consisted of an aqueous solution and acetonitrile (65:35, v/v). The aqueous solution contained 0.05% (v/v) $HClO_4$, 0.15% (w/v) 1-methane sulphonic acid and 0.25% (w/v) ammonium acetate. The eluent was monitored at 253 nm with a flow rate of 1.0 ml/min (El-Gindy et al., 2003; Krishnaiah et al., 2002). The retention times were as follows: mosapride, 5 min and trimebutine, 9.5 min.

### 2.4. Preparation of TMCT

The TMCTs were prepared using the wet granulation method. The detailed compositions of the TMCTs are given in Table 1. Trimebutine maleate, mosapride citrate and silicon dioxide pre-sieved

**Table 1**
Composition of the combination tablets of trimebutine maleate and mosapride citrate.

| Ingredients (mg/tablet) | I | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| Trimebutine maleate | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Mosapride citrate·$2H_2O$ | 5.29 | 5.29 | 5.29 | 5.29 | 5.29 | 5.29 | 5.29 | 5.29 |
| Hydroxypropylcellulose | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Silicon dioxide | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Crosscamellose sodium | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Diluent | | | | | | | | |
| D-Mannitol | 74.71 | – | – | – | 64.71 | 64.71 | 64.71 | 64.71 |
| Microcrystalline cellulose | – | 74.71 | – | – | – | – | – | – |
| Lactose anhydrous | – | – | 74.71 | – | – | – | – | – |
| Lactose monohydrate | – | – | – | 74.71 | – | – | – | – |
| Stabilizer | | | | | | | | |
| Citric acid | – | – | – | – | 10[a] | – | – | 10[b] |
| Tartaric acid | – | – | – | – | – | 10[a] | – | – |
| Succinic acid | – | – | – | – | – | – | 10[a] | – |
| Total | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |

[a] Extra-granular addition: stabilizer was added to the outside of the granule.
[b] Intra-granular addition: stabilizer was added to the inside of the granule.

through a 40 mesh screen were placed in the high shear mixer (NMG-1L, Nara, Japan). The diluents D-mannitol, microcrystalline cellulose, lactose anhydrous and lactose monohydrate, and half the amount of croscarmellose sodium, were added and mixed for 3 min. In the case of the intra-granular addition (formulation VIII), citric acid was added to this mixing step. An aqueous solution containing 25% (w/v) hydroxypropyl cellulose as a binder was added to this mixture, resulting in wet granules. These were sieved through a 10 mesh screen and dried in the oven at 60 °C for 3 h. The dried granules were passed through a 16 mesh screen and blended with the residual croscarmellose sodium and magnesium stearate. In the case of the extra-granular addition, organic acid was added to this mixing step. The TMCTs, with a diameter of 6 mm and a hardness 6–8 KP, were prepared by compressing the resulting mixtures using the ERWEKA tablet machine (GmbH, Frankfurt, Germany).

### 2.5. Stability

For the accelerated and stressed stability test, the TMCTs packaged in high-density polyethylene bottles were stored at 60 °C/75% RH for 4 weeks and their impurities were analysed at pre-determined time intervals. Furthermore, for the long-term stability test, the TMCTs packaged in HDPE bottles or Al-PVC blisters were kept at 25 °C/60% RH or 40 °C/75% RH for 6 months and their drug and impurity contents were simultaneously analysed by the HPLC method as mentioned above.

### 2.6. Thermal analysis

The thermal characteristics of pure trimebutine maleate, mosapride citrate, organic acids and binary mixtures of the drugs and organic acids were investigated using a differential scanning calorimeter (DSC-823, Mettler Toledo; Imlangacher, Greifensee, Toledo, Switzerland). The binary mixtures were prepared by physically mixing each drug and organic acid at the weight ratio of 1:1. About 4 mg of each sample was placed into a sealed aluminium pan before heating under a nitrogen flow (20 ml/min) at a heating rate of 10 °C/min from 0 to 200 °C.

### 2.7. Dissolution

The dissolution test was performed using USP XXIV, dissolution apparatus II with 900 ml pH 1.2 and pH 4.0 as dissolution media at 37 ± 0.5 °C (Choi et al., 1998). The speed of the paddle was adjusted to 50 rpm. One TMCT, a trimebutine maleate-loaded commercial tablet (100 mg trimebutine maleate) and a mosapride citrate-loaded commercial tablet (5 mg mosapride citrate) were placed into a dissolution tester (Shinseang Instrument Co., South Korea), respectively. At pre-determined intervals, 1 ml of the medium was sampled and filtered through a membrane filter (0.45 μm). The concentration of drugs in the filtrate was simultaneously analysed by the HPLC method as mentioned above.

### 2.8. Oral administration

Beagle dogs, divided into two groups, were fasted overnight and restrained by means of a dog sling (Alice King Chatham Medical Arts, Los Angeles, CA) during the 48 h experimental period. The beagle dogs were orally administered with two TMCTs or were simultaneously administered with two trimebutine maleate-loaded tablets and two mosapride citrate-loaded commercial tablets at the equivalent dose of 200 mg trimebutine maleate and 10 mg mosapride citrate, respectively. About 0.5 ml of blood was collected from the subclavian vein or artery at pre-determined time intervals. These samples were immediately centrifuged at 3000 × *g* at 4 °C for 15 min using a centrifuge (5415C; Eppendorf, Hamburg, Germany). Plasma (200 μl) was mixed with 10 μl of 8.5% (w/v) phosphoric acid to prevent trimebutine degradation and stored at −80 °C prior to analysis. To assess the individual variance in the pharmacokinetic profile, a pharmacokinetic study was conducted with an open randomized two-way crossover design with a 2-week washout period (Joo et al., 1999; Sakashita et al., 1993).

### 2.9. Blood sample treatment and HPLC/MS/MS conditions

Plasma (50 μl) was mixed with 100 μl of acetonitrile solution containing propranolol (200 ng/ml) as an internal standard. This was then centrifuged at 3000 × *g* at 4 °C for 5 min to precipitate the proteins and 5 μl of the supernatant was directly injected into the column. The plasma concentrations of trimebutine and mosapride were quantified using an Agilent 1200 LC/MS/MS system (Agilent Technologies, Palo Alto, CA, USA) equipped with an electrospray ionization interface which was used in the positive ion mode ($[M+H]^+$). The compounds were separated on a Zorbax Eclipse XDB-$C_{18}$ column (Agilent Technologies, 4.6 mm × 50 mm, 1.8 μm) with a mobile phase that consisted of acetonitrile/10 mM ammonium acetate buffer/formic acid (650:350:1, v/v/v). The column was heated to 30 °C and the mobile phase was eluted at 0.7 ml/min using an HP 1200 series pump (Agilent Technologies, Palo Alto, CA, USA) (El-Gindy et al., 2003; Krishnaiah et al., 2002). The ESI-MS data were acquired in the positive mode and the conditions of MS analysis were as follows: drying gas ($N_2$) flow rate, 8 l/min; drying gas temperature, 350 °C; nebulizing gas ($N_2$) pressure, 45 psi; capillary voltage, 3800 V; fragmentor, 100 V. Trimebutine, mosapride and propranolol (internal standard) mainly gave protonated molecules at *m*/*z* 388, 422 and 260, respectively. Furthermore, the product ions were scanned in Q3 after collision with nitrogen in Q2 at *m*/*z* 195, 198 and 116 for trimebutine, mosapride and propranolol, respectively. Quantification was performed by multiple reaction-monitoring (MRM) of the protonated precursor ions and the related product ions using the ratio of the area under the peak for each solution and a weighting factor of $1/y^2$. The analytical data were controlled by MassHunter (Agilent, Wilmington, DE, USA). The calibration curve was constructed over a range of 1–200 ng/ml in plasma ($R^2 = 0.999$) and with a lower limit of quantification (LLOQ) of 1 ng/ml for both parent drugs. For the validation, inter- and intra-day differences were conducted and the differences were found to be within an acceptable range.

### 2.10. Pharmacokinetic data analysis and statistical analysis

The area under the drug concentration time curve from zero to infinity (AUC), the elimination constant ($K_{el}$) and the half life ($t_{1/2}$) were calculated using non-compartmental analysis (WinNonlin; professional edition, version 2.1; Pharsight Co., Mountain View, CA, USA). The maximum plasma concentration of the drug ($C_{max}$) and the time taken to reach the maximum plasma concentration ($T_{max}$) were directly obtained from the plasma data (Gibaldi and Perrier, 1982). Levels of statistical significance ($p < 0.05$) were assessed using the Student's *t*-test between the two means for unpaired data. All data are expressed as the mean ± standard deviation (S.D.) or as the median (ranges) for $T_{max}$.

## 3. Results and discussion

In the present study, an attempt was made to secure a novel tablet formulation with an optimal drug substance stability. Assurance of the quality and safety of pharmaceutical products is critical and the degradation of drug substances must be suppressed to meet regulatory criteria (ICH Q3B, 2006).

First, the impurities of trimebutine and mosapride were determined for the drug substances, the trimebutine maleate-loaded

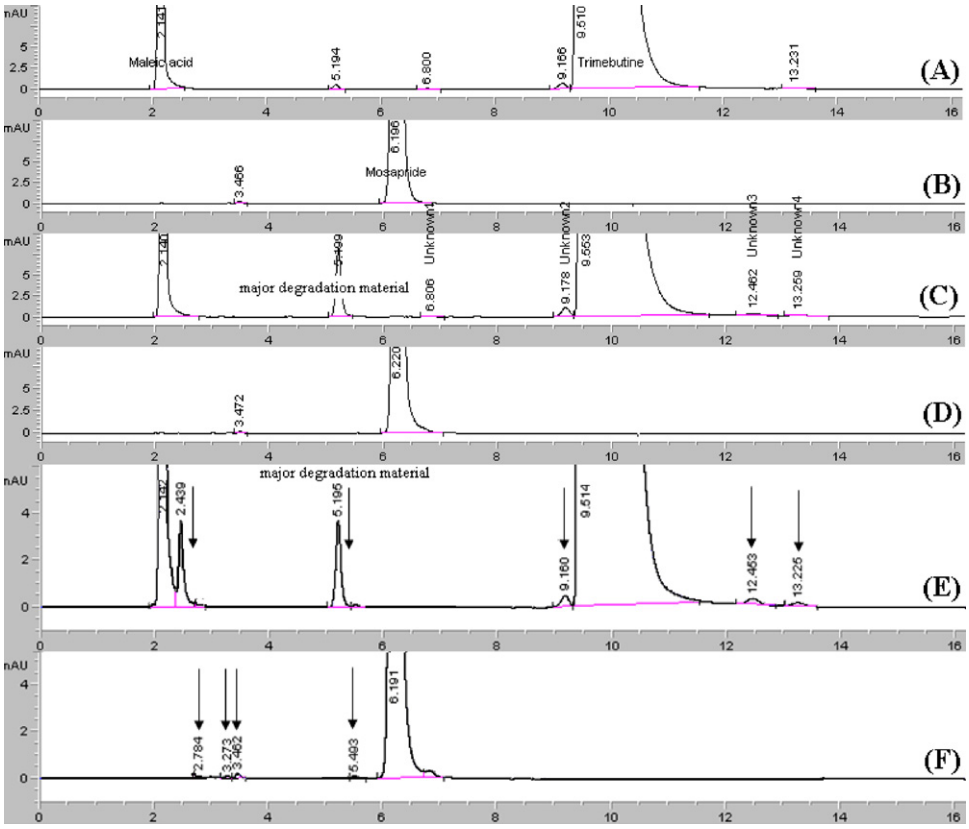


**Fig. 2.** Chromatographic specificity of impurities: (A) trimebutine maleate, (B) mosapride citrate, (C) trimebutine maleate-loaded tablet, (D) mosapride citrate-loaded tablet, (E) trimebutine maleate-loaded tablet at 40 °C/75% RH for 4 weeks, (F) mosapride citrate-loaded tablet at 40 °C/75% RH for 4 weeks.

tablet and the mosapride citrate-loaded tablet. As shown in Fig. 2(A) and (C), all the impurities of trimebutine were well separated. The trimebutine maleate-loaded tablet showed an increased level of impurities after about 5 min (major degradant) compared to drug substance. However, mosapride citrate and the mosapride citrate-loaded tablet gave no impurities (Fig. 2(B) and (D)). After accelerated storage at 40 °C/75% RH for 4 weeks, the trimebutine maleate-loaded tablet showed a further increase in the level of impurities (Fig. 2(E)). Furthermore, the mosapride citrate-loaded tablet had new four impurities, as shown in Fig. 2(F).

To evaluate the effect of diluent on the stability of the TMCT, TMCTs were prepared with the diluents D-mannitol, microcrystalline cellulose, lactose anhydrous and lactose monohydrate (Table 1, formulations I–IV). The stability of these TMCTs packaged in HDPE bottles was evaluated at the accelerated and stressed conditions of 60 °C/75% RH for 4 weeks. It was previously reported that trimebutine in an aqueous solution underwent hydrolysis of its ester bond, resulting in 3,4,5-trimethoxy benzoic acid, which is a major degradation material (Figs. 2 and 3) (El-Gindy et al., 2003; Park and Rhee, 1990). As shown in Fig. 4(A), formulation II prepared with microcrystalline cellulose gave a higher level of the major degradation products than the other formulations. Furthermore, the levels of impurities of trimebutine in formulation II were higher compared to the other formulations. As this diluent is hygroscopic, the drug in formulation II, with a relatively high humidity, was hydrolysed to a greater degree (Mwesigwa et al., 2005; Patel et al., 2003). Furthermore, formulations III and IV, prepared with lactose, showed higher levels of impurities than formulation IV (Fig. 4(B)). These formulations had increased levels of specific impurities (see the arrow in Fig. 3). These impurities might be produced by the chemical interaction between lactose and trimebutine (an amine compound) due to the Maillard reaction (Castello and Mattocks, 1962). Thus, formulations III and IV produced many impurities, even though they did not induce the production of major degradation materials. Like trimebutine, formulation I, prepared with D-mannitol, gave lower levels of mosapride impurities compared to the other formulations (Fig. 4(C)). This good stability of the drugs in the TMCTs was due to the low hygroscopicity and no chemical interactions of the amine compounds (Rowe et al., 2003). Therefore, D-mannitol was the diluent chosen for further study on the preparation of the TMCT.

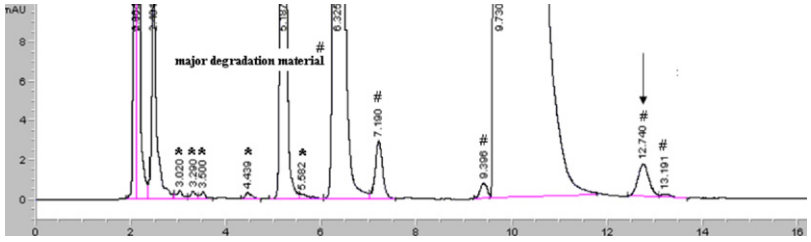


**Fig. 3.** Chromatographic selectivity of impurities at 60 °C/75% RH for 4 weeks: (*) the impurities related to mosapride, (#) impurities related to trimebutine.

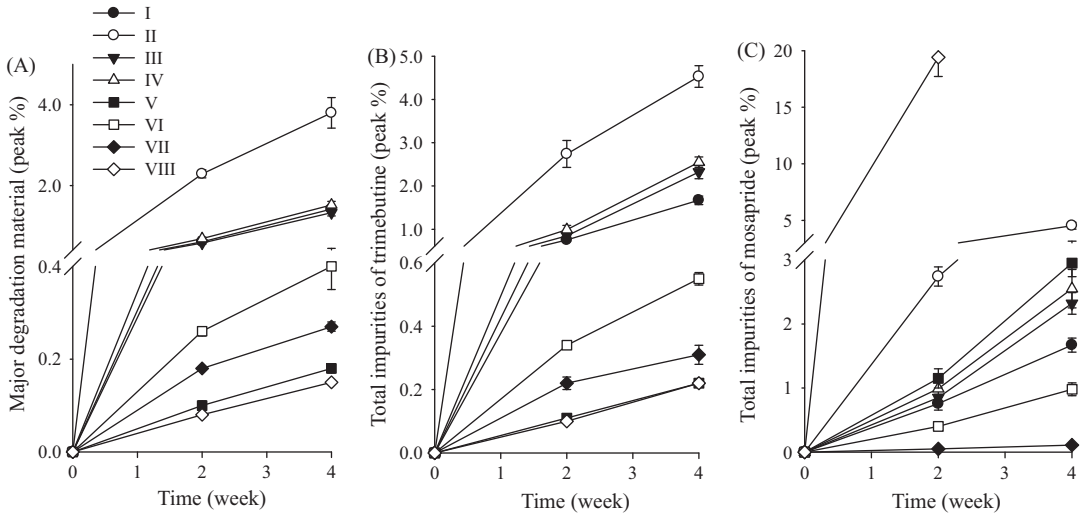


**Fig. 4.** Impurities of drugs in TMCTs at 60 °C /75% RH for 4 weeks: (A) major degradation material, (B) total impurities from trimebutine maleate, (C) total impurities from mosapride citrate. Each value represents the mean ± S.D. ($n$ = 3).

In order to test the influence of the stabilizer on the stability of TMCTs, TMCTs were prepared with the following organic acids: citric acid, tartaric acid and succinic acid (Table 1, V–VIII). The stability of TMCTs packaged in HDPE bottles was evaluated at the accelerated and stressed conditions of 60 °C/75% RH for 4 weeks. As shown in Fig. 4(A) and (B), the TMCTs prepared with organic acid (formulations V–VIII) gave fewer major degradation materials and impurities of trimebutine than the TMCTs prepared without organic acid (formulations I–IV). Furthermore, the major degradation materials and impurities of trimebutine produced from the TMCTs prepared with citric acid (formulations V and VIII) were less compared to those prepared with the other organic acids (formulations VI and VII). The levels of the major degradation materials and impurities of trimebutine in the TMCTs prepared using the extra-granular addition were lower than those which used the intra-granular addition, but there were no significant differences. Thus, among the organic acids tested, citric acid had a greater inhibitory effect on the production of major degradation materials and impurities of trimebutine. Park and Rhee (1990) reported that the hydrolysis of trimebutine in aqueous solution was considerably inhibited by citric acid. The hydrolysis of ester compounds was reduced by the micro-environmental acidic pH in the solid state (Badaway et al., 1999; Thumma et al., 2008). Moreover, succinic acid and tartaric acid were found to reduce the hydrolysis of trimebutine, even though their capacity was weaker than that of citric acid. In the present study, levels of the major degradation materials and impurities of trimebutine in TMCTs prepared with succinic acid (formulation VII) were lower than those with tartaric acid (formulation VI). Similarly, the TMCTs prepared with organic acids (formulations V–VII) using extra-granular additions produced fewer impurities of mosapride than the TMCTs prepared without organic acids (formulations I–IV) (Fig. 4(C)). However, the intra-granular addition with citric acid (formulation VIII) was not effective in inhibiting the production of mosapride impurities in the TMCTs. Among the organic acids tested, succinic acid was the most effective in suppressing the degradation of mosapride.

Fig. 5 shows the thermal behaviours of the drugs, organic acids and binary mixtures. The DSC curve shows that succinic acid and tartaric acid had an intrinsic peak at about 190 and 170 °C, respectively (Fig. 5(A) and (B)). The endothermic peaks of citric acid were observed at about 55 and 150 °C (Fig. 5(C)). Furthermore, trimebutine and mosapride showed a sharp endothermic peak at about 135 and 110 °C, corresponding to their melting points, respectively (Fig. 5(D) and (E)). For the binary mixtures of trimebutine/succinic acid (Fig. 5(F)) and trimebutine/tartaric acid (Fig. 5(G)) the intrinsic peak produced by trimebutine was not observed but instead a new peak appeared. The binary mixture of trimebutine/citric acid (Fig. 5(H)) showed no intrinsic peak of trimebutine. Furthermore, the intrinsic peak produced by mosapride was shifted to the low temperature in the binary mixtures of mosapride/succinic acid

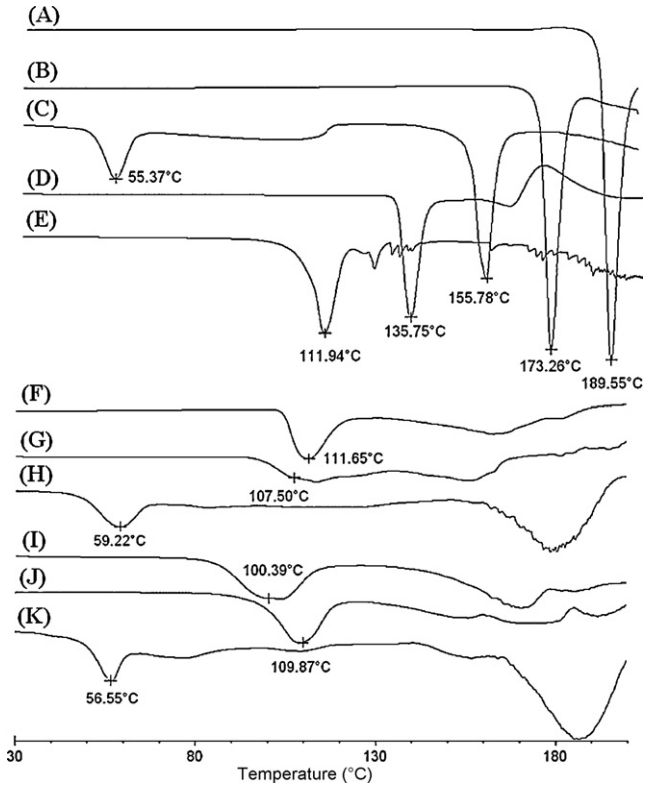


**Fig. 5.** Differential scanning calorimetric thermograms: (A) succinic acid, (B) tartaric acid, (C) citric acid, (D) trimebutine maleate, (E) mosapride citrate, (F) binary mixture of trimebutine maleate/succinic acid, (G) binary mixture of trimebutine maleate/tartaric acid, (H) binary mixture of trimebutine maleate/citric acid, (I) binary mixture of mosapride citrate/succinic acid, (J) binary mixture of mosapride citrate/tartaric acid, (K) binary mixture of mosapride citrate/citric acid. The binary mixture was composed of drug/ingredient at the weight ratio of 1/1.

**Table 2**
Long-term stability.

| Condition | Packaging | Time (month) | Assay (%) | | Major individual degradation material (peak %) | | Total impurities (peak %) | |
|---|---|---|---|---|---|---|---|---|
| | | | TB[a] | MP[b] | TB | MP | TB | MP |
| Initial | | 0 | 100.8 ± 0.1 | 97.9 ± 1.4 | 0.02 ± 0.00 | 0.06 ± 0.00 | 0.02 ± 0.00 | 0.11 ± 0.00 |
| 25 °C/60% RH | HDPE[c] | 4 | 101.0 ± 0.2 | 97.4 ± 0.9 | 0.02 ± 0.00 | 0.06 ± 0.00 | 0.02 ± 0.00 | 0.12 ± 0.00 |
| | | 6 | 102.2 ± 0.7 | 98.0 ± 0.8 | 0.03 ± 0.00 | 0.06 ± 0.00 | 0.03 ± 0.00 | 0.12 ± 0.00 |
| | Al-PVC[d] | 4 | 100.7 ± 0.3 | 97.4 ± 1.0 | 0.03 ± 0.00 | 0.06 ± 0.01 | 0.03 ± 0.00 | 0.13 ± 0.01 |
| | | 6 | 101.8 ± 0.2 | 98.0 ± 1.4 | 0.04 ± 0.00 | 0.06 ± 0.00 | 0.04 ± 0.00 | 0.12 ± 0.01 |
| 40 °C/75% RH | HDPE | 4 | 100.8 ± 0.1 | 96.9 ± 0.3 | 0.09 ± 0.00 | 0.08 ± 0.01 | 0.11 ± 0.01 | 0.18 ± 0.01 |
| | | 6 | 101.8 ± 0.3 | 97.1 ± 1.2 | 0.14 ± 0.01 | 0.07 ± 0.01 | 0.16 ± 0.01 | 0.15 ± 0.01 |
| | Al-PVC | 4 | 99.4 ± 0.1 | 96.8 ± 0.2 | 0.11 ± 0.00 | 0.06 ± 0.01 | 0.12 ± 0.01 | 0.13 ± 0.01 |
| | | 6 | 101.1 ± 0.4 | 97.3 ± 0.3 | 0.09 ± 0.01 | 0.07 ± 0.01 | 0.22 ± 0.01 | 0.14 ± 0.01 |

Each value represents the mean ± S.D. ($n = 3$).
[a] Trimebutine maleate.
[b] Mosapride citrate.
[c] High-density polyethylene bottle.
[d] Aluminum polyvinylchloride blister.

(Fig. 5(I)) and mosapride/tartaric acid (Fig. 5(J)). As with the binary mixture of trimebutine/citric acid (Fig. 5(H)), the binary mixture of mosapride/citric acid (Fig. 5(K)) showed no intrinsic peak of mosapride. Our results suggest that these drugs interacted with the organic acids (Li et al., 2010). In particular, the binary mixtures of the drugs and citric acid gave a peak at about 55 °C and a lower endothermic change compared to succinic acid and tartaric acid. Thus, the direct exposure of citric acid to mosapride by the intragranular addition might accelerate the degradation of mosapride at about 55 °C due to an endothermic change such as melting at the accelerated and stressed conditions of 60 °C/75% RH (Yoo et al., 2009).

From the results of the accelerated and stressed stability test, TMCT prepared with succinic acid gave the lowest level of total impurities of the drugs at 60 °C/75% RH for 4 weeks. Therefore, succinic acid was chosen as the stabilizer for further study on the preparation of the TMCT.

For long-term stability, the TMCTs were manufactured with D-mannitol and succinic acid using extra-granular addition via pilot scale batch sizes. These TMCTs were packaged in HDPE bottles or Al-PVC and kept for 6 months under two different conditions (Table 2). We evaluated stability from the contents of drugs and impurities in the TMCTs after 6 months. The contents of trimebutine and mosapride were higher than 95% and decreased less than 1% at 25 °C/60% RH and 40 °C/75% RH during the 6-month period, indicating that there was no noticeable change in the drug contents. Furthermore, these TMCTs gave less than 0.5% of the total impurities of mosapride and trimebutine. Generally, the acceptable level of active drug impurities in conventional products is less than 0.5% (Greenlees, 2003; Kroes and Kozianowski, 2002). Thus, the TMCTs packaged in HDPE bottles or Al-PVC were stable for at least 6 months.

The dissolution studies on the TMCT, the trimebutine maleate-loaded commercial product and the mosapride citrate-loaded commercial product were performed in 0.1N HCl (pH 1.2) and acetate buffer solution (pH 4.0). The dissolution profiles of these three preparations are shown in Fig. 6. The amounts of trimebutine dissolved from the TMCT were similar to those of the trimebutine maleate-loaded commercial product at pH 1.2 and pH 4.0. Similarly, as shown in Fig. 6(A) and (B), there were no significant differences between the amounts of mosapride dissolved from the TMCT and the mosapride citrate-loaded commercial product; they showed almost complete dissolution at pH 1.2 and pH 4.0 within 30 min.

The in vitro dissolution profiles of the TMCT and the commercial products were compared using the difference factor ($f_1$) and similarity factor ($f_2$), as defined by the following equations (Cao et al., 2005; Hernandez et al., 1994):

$$f_1 = \left[ \frac{\sum(R_t - T_t) / \sum(R_t + T_t)}{2} \right] \times 100 \quad (1)$$

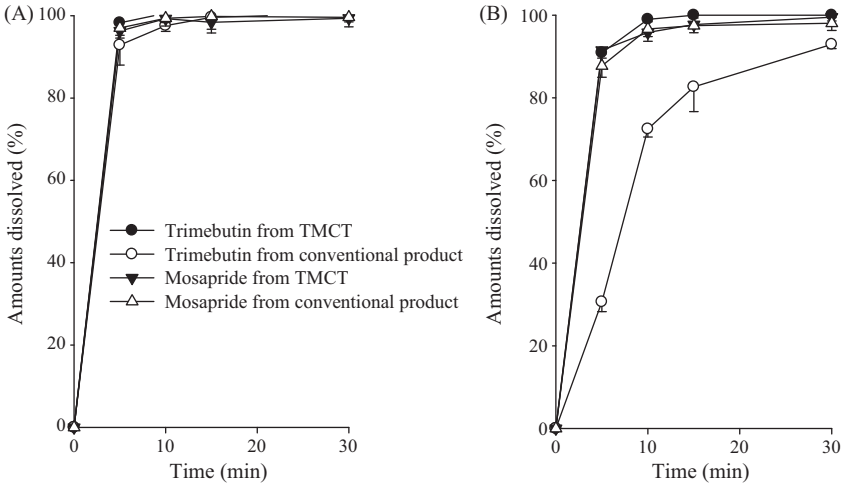


**Fig. 6.** Dissolution of trimebutine maleate and mosapride citrate from TMCT and conventional products at pH 1.2 (A) and pH 4.0 (B). Each value represents the mean ± S.D. ($n = 6$).

**Table 3**
Difference factors and similarity factors between the TMCTs and the commercial products.

| Dissolution medium | pH 1.2 | | pH 4.0 | |
|---|---|---|---|---|
| | Trimebutine | Mosapride | Trimebutine | Mosapride |
| Difference factor ($f_1$) | 2.39 | 0.64 | 1.45 | 1.64 |
| Similarity factor ($f_2$) | 74.20 | 94.37 | 83.24 | 81.99 |

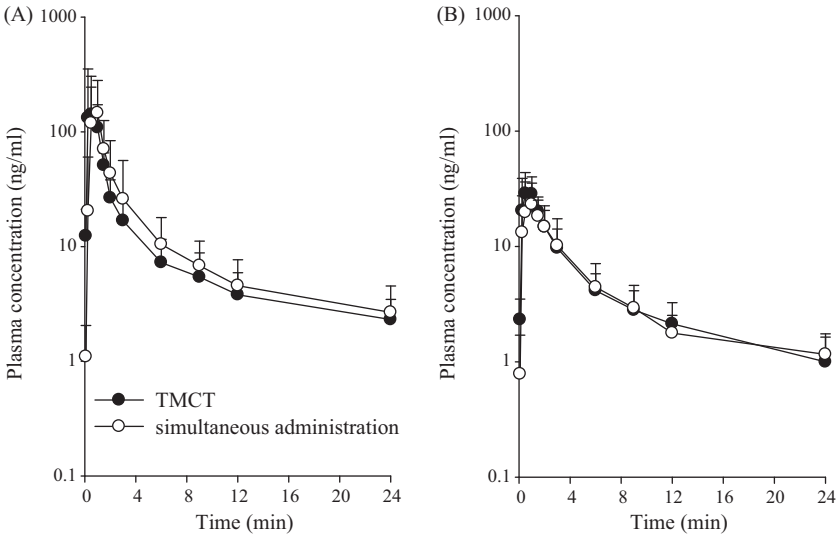


**Fig. 7.** Blood concentration–time profiles of trimebutine maleate and mosapride citrate after oral administration of TMCT or simultaneous oral administration of trimebutine maleate-loaded and mosapride citrate-loaded commercial products at the equivalent dose of 200 mg trimebutine maleate and 10 mg mosapride citrate in the beagle dog: (A) trimebutine maleate, (B) mosapride citrate. Each value represents the mean ± S.D. ($n = 8$).

$$f_2 = 50 \times \log\left\{\left[1 + \frac{1}{n}\sum (R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (2)$$

where $n$ is the number of time points, and $T_t$ and $R_t$ are the amounts dissolved at the time point ($t$) for the TMCT and the commercial products, respectively. In these equations, $0 < f_1 < 15$ and $50 < f_2 < 100$ mean that a similar correlation was found between the dissolution patterns of the two products. As shown in Table 4, the difference factor ($f_1$) values between the TMCT and the trimebutine maleate-loaded commercial product were 2.39 and 1.45 at pH 1.2 and 4.0 for trimebutine maleate, respectively. They had similarity factor ($f_2$) values of 74.20 and 83.24 at pH 1.2 and 4.0, respectively. Furthermore, the TMCT and the mosapride citrate-loaded commercial product gave difference factor ($f_1$) values of 0.64 and 1.64 at pH 1.2 and 4.0, and similarity factor ($f_2$) values of 94.37 and 81.99 at pH 1.2 and 4.0, respectively (Table 3). Thus, the TMCT and the commercial products showed similar correlations of dissolution profiles at pH 1.2 and pH 4.0.

Fig. 7 shows the change in mean plasma concentration of trimebutine (A) and mosapride (B) after oral administration of the TMCT or simultaneous administration of trimebutine maleate-loaded and mosapride citrate-loaded commercial products at the equivalent dose of 200 mg trimebutine maleate and 10 mg mosapride citrate in beagle dogs. The total plasma concentrations of trimebutine and mosapride in the TMCT were not significantly different from those in the commercial products in beagle dogs.

The pharmacokinetic parameters are shown in Table 4. The AUC, $C_{max}$ and $T_{max}$ values of trimebutine and mosapride for the TMCT did not significantly differ from the commercial products. Furthermore, the $K_{el}$ and $t_{1/2}$ values of trimebutine and mosapride from the TMCT were not significantly different from those of the commercial products. Thus, from the pharmacokinetic point of view, this TMCT showed a similar drug efficacy compared to the commercial products in beagle dogs. This combination tablet of trimebutine maleate and mosapride citrate might increase patient compliance and control the complex symptoms of functional dyspepsia easily.

**Table 4**
Pharmacokinetic parameters.

| | TMCT | Simultaneous administration[a] |
|---|---|---|
| Trimebutine maleate | | |
| AUC (ng h/ml) | 352.0 ± 134.8 | 424.5 ± 240.2 |
| $C_{max}$ (ng/ml) | 244.8 ± 219.1 | 281.1 ± 263.6 |
| $T_{max}$ (h) | 0.5 ± 0.2 | 0.8 ± 0.4 |
| $K_{el}$ ($h^{-1}$) | 0.4 ± 0.3 | 0.4 ± 0.7 |
| $t_{1/2}$ (h) | 3.4 ± 5.8 | 7.7 ± 6.7 |
| Mosapride citrate | | |
| AUC (ng h/ml) | 129.3 ± 77.8 | 124.3 ± 57.3 |
| $C_{max}$ (ng/ml) | 39.4 ± 14.6 | 35.3 ± 11.3 |
| $T_{max}$ (h) | 0.6 ± 0.2 | 0.8 ± 0.5 |
| $K_{el}$ ($h^{-1}$) | 1.6 ± 1.7 | 1.2 ± 1.1 |
| $t_{1/2}$ (h) | 0.6 ± 0.2 | 3.1 ± 5.5 |

Each value represents the mean ± S.D. ($n = 8$).
[a] simultaneous oral administration of trimebutine maleate-loaded and mosapride citrate-loaded commercial products.

## 4. Conclusion

In conclusion, the combination tablet of trimebutine maleate and mosapride citrate prepared with D-mannitol as the diluent and succinic acid as the stabilizer was stable for at least 6 months. Furthermore, it showed similar dissolution to the trimebutine maleate-loaded and mosapride citrate-loaded commercial products, and gave insignificantly different absorption compared to these commercial products in beagle dogs. Thus, this combination tablet would be a stable and effective oral pharmaceutical product for the treatment of functional dyspepsia. For the development of

a novel trimebutine maleate and mosapride citrate-loaded combination tablet, further bioequivalence tests in human subjects will be performed.

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