Evidence for the Degradation of Maleate Moiety in Chlorpheniramine Maleate Solution Using a Stability-Indicating HPLC Method

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The degradation phenomenon of maleate moiety of chlorpheniramine maleate in solution has been demonstrated by means of a peculiar ion-pair HPLC method developed by the authors, which permits the simultaneous determination of chlorpheniramine and maleate. A commercial cough drug containing chlorpheniramine maleate was dissolved in water with m-hydroxybenzoic acid as an internal standard, and then kept for several days at room temperature. It was recognized that the maleate content in the drug solution had gradually decreased, whereas chlorpheniramine content had not decreased. A simple solution of maleic acid was also kept for several days at room temperature, and it was also recognized that the maleate content in the solution preserved at the same concentration as the solution of the commercial cough drug had gradually decreased, and the percent of remaining maleate reached zero. The degraded peaks on HPLC chromatograms were not detected at all by UV detector, and the disappearance of maleate was ascertained by GC-MS. No detectable example of maleate of chlorpheniramine maleate in a commercial cough syrup has suggested that maleate moiety of chlorpheniramine maleate decomposed to carbon dioxide.

Key words: maleate degradation; chlorpheniramine maleate; stability-indicating HPLC method

Chlorpheniramine maleate (CPM) is a highly potent antihistamine widely used for the symptomatic treatment of common colds and allergic diseases. Its pharmaceutical formulation on the market varies, and it is found as tablets, granules, capsules and syrup. 1

High-performance liquid chromatography (HPLC) is a method used exclusively for the analysis of pharmaceutical preparations with dosage forms. The common mode for the quantitative determination of CPM has been the reversed-phase ion-pair HPLC using an anionic counter-ion such as heptanesulfonic acid, pentanesulfonic acid or diocetyl sulfo-

uncin acid. 2–4 It is naturally essential that chlorphenira-

mine (CP), a weak basic moiety, is ion-paired with an-

ionic counter-ions. On the contrary, we have developed a peculiar ion-pair HPLC using a cationic counter-ion, tetra-n-

butyl ammonium phosphate (TBAP), which permits the simultaneous determination of CP and maleic acid (MA), a weak acidic moiety. 5 This method was successfully applied to the determination of CPM in commercial ophthalmic solutions 5 and in cough and cold drugs. 6 Five other antihista-

mines analogous to CPM were also separable by this method. 6

The easy simultaneous determination of CP and MA, i.e. CPM analysis, plays a role not only for quantitative analysis and identification but also for purity test. We have proposed this method as being very useful in the quality control of manufactured CPM.

The proposed method was applied to the simultaneous de-

termination of CP and MA, i.e. CPM analysis, in commercial cough and cold drugs; their pharmaceutical forms were one tablet, one granule and three syrups. The interesting result observed was that MA was not detected in one product among three commercial cough syrups, despite the identifi-

cation of CP in that product, which was consistent with the label. 9 Consequently, a further search was made employing this peculiar HPLC method to elucidate the cause. In the pre-

sent report the decomposition phenomenon of MA moiety of CPM in an extemporaneously prepared liquid solution is investigated. The need for the simultaneous determination of CP and MA for the practical quality control of manufactured CPM is also discussed together with the acid’s stability in solution.

Materials and Methods

Materials m-Hydroxybenzoic acid (m-HOBA) was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). p-Amino benzoic acid (p-ABMA) and maleic acid (MA) were purchased from Wako Pure Chemical Industries (Osaka, Japan). The ion-pair reagent, tetra-n-butyl ammonium phosphate (TBAP), was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Bis(trimethylsilyl) trifluoroacetic acid (BSTFA) was a product of Tokyo Kasei Kogyo Co., Ltd. HPLC-grade methanol was obtained from Kanto Chemical Co. Inc. (Tokyo). Cough drug was purchased from the market. Stock and working standard solutions of m-HOBA and p-ABMA were prepared according to a previous paper. 9

Preparation of Sample Solutions for Stability Studies

Granules in the cough capsule were placed in a beaker and a given amount was weighed. The granules were thoroughly ground in a mortar with a pestle. The powdered sample was dissolved in doubly demineralized, distilled water, m-HOBA as an internal standard (IS) was added, and then adjusted to the required concentrations. The solution was centrifuged at 2000 rpm for 15 min to remove insoluble materials, and then the supernatant was filtered through a 5-μm pore size filter. Final concentrations of CPM and the IS were 50 μg/ml (0.128 μm) as the labeled content and 0.2 μm, respectively. A simple MA solution was prepared by dissolving MA in doubly demineralized, distilled water. p-AMBA instead of m-HOBA was used as the IS. Final concentrations were 0.1 μm and 0.2 μm for MA and the IS, respectively. Each solution of CPM and MA was put into 10-ml brown-glass containers with Teflon-lined closures, and placed in a room with a temperature of 23–29 °C and humidity of 60–80%. An aliquot of each sample solution was injected into the HPLC at certain time intervals.

HPLC System and Analytical Procedures

The chromatographic system and analytical conditions were as described previously. 10 In brief, a Shimadzu (Kyoto) LC-6A pump, equipped with a Rhodyne (Cotati, CA, U.S.A.) Model 7125 syringe-loading sample injector with 100-μl sample loop, a Capcell Pak C18 analytical column (6 mm i.d.×150 mm, SG type, 5-

μm particle; Shiseido, Tokyo) and a Shimadzu CTO-6A column oven maintained at 30 °C were used respectively. The mobile phase consisted of a mix-

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ture of ion-pair reagent (TBAP), potassium dihydrogen phosphate (KH₂PO₄) and methanol, and the pH was adjusted to 3.0 with 5% orthophosphoric acid (H₃PO₄). The mobile phase was filtered through a 0.4-μm pore size filter (Fuji Photo Film, Tokyo), and was pumped at a flow-rate of 1 ml/min. The samples of solutions of 10-μl were injected through a Rheodyne injector. Effluents obtained with isocratic elution were monitored at 215 nm with a Shimadzu SPD-6AV spectrophotometer equipped with an 8-μl flow cell. Chromatographic data were recorded on a CR-4A integrator (Shimadzu).

**GC-MS Analysis** Five hundred milliliters of 0.1 mg MA solution was kept for 7 d at room temperature, and then lyophilized. The residue was collected and suspended in a small volume of chloroform, and placed in a small reaction vessel. The solvent was evaporated in vacuo, and the dried residue was dissolved in 100-μl of chloroform. The volatile trimethylsilyl (TMS) derivatization was carried out by adding 10-μl of BSTFA, and the mixture was allowed to stand for 60 min at 60°C. One microliter of the reaction mixture was injected into the gas chromatograph-mass spectrometer (GC-MS) under the following conditions: GC-MS, Hewlett Packard 5890 Series II gas chromatograph connected to a Model 5971 A mass detector; detection mode, electron impact (EI); ionization potential, 70 eV; column, capillary column of HP-1 (crosslinked with methyl silicone, 0.2 mm i.d.×12 m); carrier, helium gas at a flow-rate of 0.8 ml/min; injector temperature, 250°C; ion source temperature, 180°C; interface temperature, 280°C; programming of column temperature, left for 2 min with an initial temperature at 70°C, this was increased at a rate of 10°C/min to 240°C, and maintained for 11 min.

**Results and Discussion**

Stability-indicating HPLC method is one of the conventional techniques for determining the degradation rate of drugs. Yacobi et al. reported the results of stability testing for pseudoephedrine hydrochloride and CPM in pharmaceutical dosage forms using this technique. Stability of enalapril maleate, used for the treatment of hypertension and congestive heart failure, in extemporaneously prepared oral liquid has also been studied using the stability-indicating HPLC method. Although the stability of these drugs was examined for their weak basic moieties, no reports could be found describing the stability characteristics of the weak acidic moiety, MA. Let us reconsider the data which appeared in the previous paper. The HPLC method developed by the authors was successful in separating CP and MA from other active and inactive ingredients, however, one of the pharmaceutical preparations, a sample with the formulation of syrup, did not allow determination of MA. Despite this, the found and labeled content of CP in the syrup formulation were concordant. The data have been shown in Fig. 3 and Table 1 in ref. 6. We have tried to prove this phenomenon experimentally. Granules in a commercial cough capsule with a simple ingredient containing CPM were extemporaneously dissolved in water, and then the CPM solution was stored at ambient temperature. A noteworthy result observed was the decomposition behavior of MA in CPM (Fig. 1). The proportion of CP to the IS, m-HOBA, was not changed, whereas that of MA to the IS was reduced with the course of time. From the chromatographic data, the degradation rates of MA were calculated as about 48% and 80% after allowing the acid to stand for 1 week and 2 weeks, respectively. It has been reported that CP is stable at various pH values and under moderate temperature conditions, however, no description concerning the stability of MA can be found in the literature. To further clarify this phenomenon of the decomposition of MA, a simple MA solution was kept for several days at room temperature. The constitution of the mobile phase and the IS were changed for reduction in analytical time. The IS of p-AMB instead of m-HOBA was just appropriate. Figure 2 indicates that a peak of MA in the sample solution preserved at the concentration of 0.1 mg, the same concentration as the CPM solution of the previous experiment, had gradually decreased with the passage of time. The proportion of MA to the IS, p-AMB, was plotted as a function of time (Fig. 3). It is apparent that MA in the sample solution had gradually decreased over time, and the peak of MA had completely disappeared after 5 days. Degraded peaks on HPLC chromatogram were not detected, with the exception of a small peak identified as fumaric acid (FA) sometimes (Fig. 2A). A trace amount of MA and its possible metabolites remaining in the solution after the decomposition of MA were also ascertained by GC-MS. Under the experi-
Fig. 3. The Degradation of MA in Solution as a Function of the Time-Course

A simple solution of MA with p-AMBA as IS was allowed to stand for several days at ambient temperature. Final concentrations were 0.1 mS and 0.2 mS for MA and the IS, respectively. The ratio of MA vs. IS is plotted as a function of the time-course.

Experimental conditions, no products assigned a significance were yielded, nor were any peaks of retention time identical to that of the authentic standards of MA, FA, malic, succinic and tartaric acids detected by monitoring of the total ion mode. The production of MA by catalytic oxidation of benzene and other possible conversions of MA, i.e., isomerization, hydration, oxidation, reduction or the decomposition to carbon dioxide and water, etc. were described in the literature.[11—13]

From the results of the HPLC chromatogram and GC-MS data described above, MA and FA should decompose to carbon dioxide, even though only a small amount of MA was temporarily isomerized to FA. In conclusion, the present results suggest that no detectable example of MA of CPM in a commercial cough syrup results from decomposition of the MA moiety on storing. The simultaneous determination of CP and MA, especially the MA offers another piece of information on the past histories of the drugs. As for the detailed mechanism of the degradation, we shall wait to see what the future holds.

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References