Fractional Factorial Experimental Design Study of the Incompatibility of Benzocaine in Throat Lozenges

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Abstract □ The instability of benzocaine in a throat lozenge formulation has been shown to be due to the reactivity of the primary aromatic amine group. Three common excipients of throat lozenges were found to be incompatible with benzocaine.

Keyphrases □ Lozenges—benzocaine instability □ Benzocaine incompatibility—lozenge excipients □ Bratton and Marshall reaction—analysis □ Fractional factorial design—benzocaine incompatibility

A formulation-development program for a throat lozenge containing benzocaine was undertaken. A study of the classical literature reference sources such as "Husa's Pharmaceutical Dispensing," "Remington's Pharmaceutical Sciences," and "United States Dispensatory" did not indicate any stability problems except for the well-documented (1–3) ester hydrolysis in aqueous media. Since a throat lozenge is a solid dosage form, no problems from this source were envisioned.

It was thus very unexpected when the initial assay of a freshly prepared throat lozenge indicated that the benzocaine content was only about 20% of the formulated strength. This paper covers the investigation undertaken to determine the causes of this incompatibility problem.

EXPERIMENTAL

Preparation of Throat Lozenges—The basic throat lozenge formula is listed in Table I.

The benzocaine, cetylpyridinium chloride, citric acid, hexylresorcinol, menthol, and sugar were thoroughly mixed and passed through a comminuting mill to insure uniformity. The mixture was granulated with a warm solution of corn syrup (containing the dye and flavors).

The resulting damp granules were passed through a No. 8 mesh screen, trayed, and dried at 55° for about 16 hr. The dried granulation was then passed through a No. 16 mesh screen. After thoroughly mixing with magnesium stearate and starch, the granulation was compressed into lozenges weighing 2.00 g.

Table I—Benzocaine Throat Lozenge Formula

Component	mg.
Benzocaine	5.0
Cetylpyridinium chloride	2.0
Citric acid	10.0
Corn syrup	50.0
F.D.C. Red No. 2	1.0
Hexylresorcinol	1.0
Imitation mint flavor	3.0
Magnesium stearate	2.5
Menthol	4.0
Natural cherry flavor	100.0
Starch	45.0
Sugar	1,776.5
Total	2,000.0

Analytical Procedure—The lozenge was placed in a 50-ml. centrifuge tube containing 50 ml. of pH 4.5 acetate buffer. The centrifuge tube was shaken for 30 min. during which time the lozenge was completely dissolved. After the solution was centrifuged, the standard Bratton and Marshall reaction (4, 5) was run on a 2-ml. aliquot of the aqueous phase.

Experimental Design—Three sets of two-level fractional factorial experiments (6) were performed on the formulation (Table I). The factors studied were either omitted (− level) from the formulation or present (+ level) at the appropriate level. The composition of the formulas in the A Study, B Study, and C Study are listed in Table II, Table III, and Table IV, respectively. When the sugar was omitted during the A Study, talc was substituted to maintain the size of the lozenge.

Statistical Treatment of Data—The fractional factorial experimental designs were analyzed by the method of Yates (7).

RESULTS AND DISCUSSION

Throat lozenges containing 2 mg. of benzocaine were prepared and submitted to a taste panel which noted a low level of anesthetic sensation for the product. The benzocaine content of successive lozenge formulations was gradually increased to 5 mg. These also lacked the anesthetic sensation, and thus were submitted for chemical analysis. The benzocaine content of these freshly prepared throat lozenges was about 20% of the formulated strength (using a Bratton-Marshall reaction which was dependent on the presence of the primary aromatic amine group of the benzocaine molecule).

The possible causes of benzocaine incompatibility were investigated by the use of two-level fractional factorial experimental designs. The first set of experimental formulations (A Study) involved only three of the eleven variables. This was intentionally limited in order to verify that the original formulation was not an outlier. The three factors, magnesium stearate, starch, and sugar (Table II), were found not to contribute significantly (Table V) to the incompatibility problem. The average benzocaine content of these throat lozenges was about 25% of the formulated strength.

Since the A Study indicated that the incompatibility problem was real, a second experiment was undertaken (B Study). This time seven new factors, cetylpyridinium chloride, citric acid, F.D.C. Red No. 2, hexylresorcinol, imitation mint flavor, menthol, and natural cherry flavor, were studied. A saturated 2^{7-4} fractional factorial experimental design (Table III) consisting of seven variables in eight formulations was used. The results (Table VI) indicated that three of the excipients, citric acid, F.D.C. Red No. 2, and natural cherry flavor, were major causes for the incompatibility problem. When only these three excipients were absent, 73% of the formulated benzocaine content was found. While when all seven factors were absent, again only 74% of the formulated benzocaine content was found. This indicated that the remaining eleventh excipient, which

Table II—Design and Benzocaine Content of Throat Lozenges in A Study

Formulation[a]	A-1	A-2	A-3	A-4
Level of factor				
Magnesium stearate	+[b]	−[c]	−	+
Starch	−	−	+	+
Sugar	−	+	−	+
Benzocaine content found, mg.	1.14	1.23	1.16	1.48

[a] All other excipients listed in Table I present. [b] + means that factor is present. [c] − means that factor is not present.

Table III—Design and Benzocaine Content of Throat Lozenges in B Study

Formulation[a]	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8
Level of Factor								
Cetylpyridinium chloride	−	+	−	+	−	+	−	+
Citric acid	−	+	+	−	+	−	−	+
F.D.C. Red No. 2	−	+	−	+	+	−	+	−
Hexylresorcinol	−	+	+	−	−	+	+	−
Imitation mint flavor	−	−	−	−	+	+	+	+
Menthol	−	−	+	+	+	+	−	−
Natural cherry flavor	−	−	+	+	−	−	+	+
Benzocaine content found, mg.	3.71	1.15	1.34	0.98	0.98	3.66	1.00	1.17

[a] All other excipients listed in Table I present.

had not been studied, also was contributing to the incompatibility problem. The average benzocaine content of the B Study was 35% of the formulated strength.

A new set of factorial experiments (C Study) was undertaken. This time a 2^{4-1} fractional factorial experiment was undertaken including the three incompatible factors and the unstudied variable, corn syrup, four factors and eight formulations in all. The average benzocaine content of the C Study formulations was 70% of label strength. The incompatibility of only two (citric acid and natural cherry flavor) out of the three factors (Table VII) found in the B Study was confirmed. The third factor (F.D.C. Red No. 2) had shown up in the B Study due to the interactions which were confounded. The eleventh factor, corn syrup, was also found to contribute to the incompatibility problem. When all four of these excipients were absent in the formulation, the benzocaine content was 93% of the formulated strength, a significant rise over the 74% value found in the B Study (where the eleventh factor was still present). A taste panel noted an adequate level of anesthetic sensation for this product. A modification of the assay procedure permitting separation of any possible *p*-aminobenzoic acid (extraction of pH 8.0 aqueous solution with benzene) yielded similar benzocaine results indicating that the ester group was still intact.

A study of the chemical literature revealed the probable reasons for the incompatibility of the three excipients, citric acid, corn syrup, and natural cherry flavor, with benzocaine.

Higuchi *et al.* (*8*) have reported the reversible formation of amides from polycarboxylic acids and aromatic amines in aqueous solution (optimum conditions at a pH of *ca.* 4). In particular they studied the reaction between benzocaine and citric acid in an aqueous system at 95°. The aryl amine disappeared relatively rapidly (*ca.* 10 hr. at pH 4.0) but soon reached an equilibrium concentration. They were able to isolate a reaction product whose properties corresponded to the monoamide. This compound on heating at 95° in water rapidly reverted (*ca.* 1 hr. at pH 3.5) to the free acid and benzocaine. Higuchi (9) showed further that the role of the polycarboxylic acid on the formation and hydrolysis of certain amido acids was mediated by the formation of acid anhydrides. In a third paper (10), a detailed study of the rate of interaction of citrate buffer with aniline to form anilides (I) and imides (II) was given. The speed and nature of the reaction suggested that these interactions may be responsible for the loss of activity of some drugs formulated with citrate buffer. The equilibrium and reactions involved appear to be rather complex but may be represented essentially by Scheme I.

Table IV—Design and Benzocaine Content of Throat Lozenges in C Study

Formulation[a]	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
Level of Factor								
Citric acid	−	+	−	+	−	+	−	+
Corn syrup	−	+	+	−	+	−	−	+
F.D.C. Red No. 2	−	−	+	+	−	−	+	+
Natural cherry flavor	−	−	−	−	+	+	+	+
Benzocaine content found, mg.	4.63	3.13	3.63	4.19	3.19	2.81	2.97	3.16

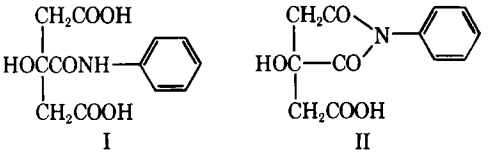
[a] All other excipients listed in Table I present.

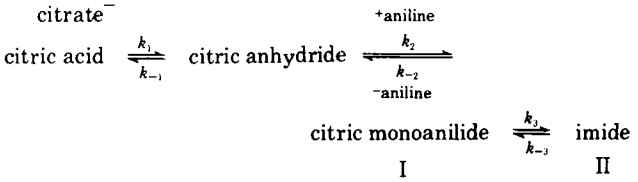
Table V—Calculation of Effects in A Study

Factor	Estimated Effect, × 2
Magnesium stearate	+0.23[a]
Starch	+0.27[a]
Sugar	+0.41[a]

[a] Not significant.

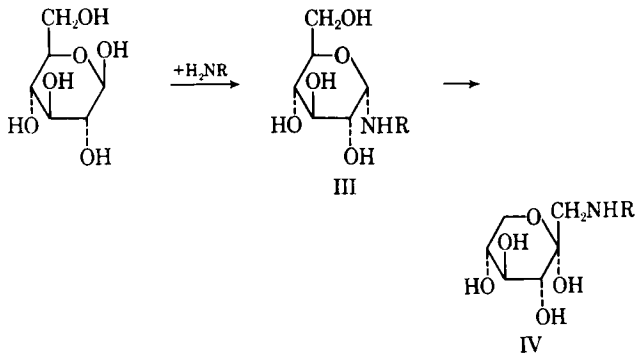
Corn syrup is incompatible with benzocaine mainly because of its glucose content. Glucose reacts with primary aromatic amines (11) to yield *N*-arylglycosylamines (*N*-glycosides, III, and not Schiff





Scheme I

bases). This condensation (Scheme II) proceeds best in the presence of a small quantity of water at pH 3–4. These compounds are labile and undergo hydrolysis as well as the Amadori rearrangement to form amino ketones called isoglucosamines (IV). The isoglucosam-



Scheme II

ines do not hydrolyze. Glucosylarylamines (12), even in acid (*N* H_2SO_4) solution, hydrolyze very slowly.

The reactivity of aromatic amino compounds is used for the colorimetric determination (11) of sugars, notably aldoses. Aniline is employed in conjunction with various organic acids such as acetic, oxalic, trichloroacetic, and phthalic acids. *p*-Aminobenzoic acid has been used as a colorimetric reagent to determine glucose (13) in body fluids. The same reagent with oxalic acid has been used by Roy (14) for detecting sugars on paper chromatograms. Leopold (15) recently improved the colorimetric determination of sugars using phosphoric acid with *p*-aminobenzoic acid.

Table VI—Calculation of Effects in B Study

Factor	Estimated Effect, × 4
Cetylpyridinium chloride	−0.07
Citric acid	−4.71[a]
F.D.C. Red No. 2	−5.77[a]
Hexylresorcinol	+0.31
Imitation mint flavor	−0.37
Menthol	−0.07
Natural cherry flavor	−5.01[a]

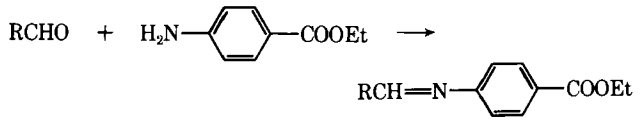
[a] Significant.

Table VII—Calculation of Effects in C Study

Factor	Estimated Effect, × 4
Citric acid	−1.13[a]
Corn syrup	−1.49[a]
F.D.C. Red No. 2	+0.19
Natural cherry flavor	−3.45[a]

[a] Significant.

Natural cherry flavor is incompatible with benzocaine probably because of the presence of natural reducing sugars (glucose, *etc.*) and aldehydes. The latter react with benzocaine to produce Schiff bases (16, 17) as illustrated in Scheme III.



Scheme III

SUMMARY AND CONCLUSIONS

Benzocaine in a throat lozenge formulation with 11 excipients was found to be unstable. Fractional factorial experiments identified three excipients, citric acid, corn syrup, and natural cherry flavor, as the causes of the incompatibility. The primary aromatic amine grouping instead of the ester linkage of benzocaine was involved in the stability problem. The standard pharmaceutical literature reference sources do not usually list the extent of benzocaine's incompatibility with commonly used pharmaceutical excipients.

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Synthesis and Antitussive Activity of *N*-[Indenyl(3)]ureas

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Abstract □ Starting from indene, a variety of *N*-[indenyl(3)] ureas were synthesized and their pharmacological properties investigated. Several members of the series showed potent antitussive properties but also were found to possess a high order of toxicity.

Keyphrases □ *N*-[Indenyl(3)]ureas—synthesis □ Antitussive activity—*N*-[indenyl(3)] ureas □ UV spectrophotometry—structure □ IR spectrophotometry—structure □ NMR spectroscopy—structure

Iodine isocyanate has been used successfully in recent years for the stereospecific synthesis of β-iodo isocyanates from olefins (1–3). Hassner and Heathcock (4,5) have shown that the addition of iodine isocyanate occurs in a stereospecific fashion and that iodine and the isocyanate functions are introduced *trans* to each other and diaxially in rigidly fused cyclohexanes. The above authors have also shown that methyl-*N*-[*trans*-2-iodo-1-indanyl] carbamate could be pyrolyzed to *cis*-indano [2,1-*b*]-2-oxazolidone. It was also shown that the above carbamate, in presence of base at room temperature or at slightly elevated temperature, is cyclized to an aziridin derivative with concomitant formation of indan-1-one as byproduct (6). *trans*-2-Iodocyclohexyl isocyanate was prepared by Wittekind *et al.* (2), converted to the corresponding urea by treatment with ammonia, and cyclized in presence of base to *cis*-2-amino-3*a*,4,5,6,7,7*a*-hexahydrobenzoxazole. This aminooxazoline was found to be a long-acting sympathomimetic agent. It is also known that some 2-amino indane derivatives synthesized by Huebner *et al.* (7) show analgesic activity. The possibility of synthesis of compounds of pharmacological interest prompted the authors to examine the prod-