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GAS CHROMATOGRAPHIC DETERMINATION OF ISBUFYLLINE IN TABLETS FOR STABILITY PURPOSES

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Abstract

Isbufylline is a new antibronchospastic drug.

A rapid and simple gas chromatographic assay of isbufylline in tablet matrices has been developed for stability purposes. Isbufylline was extracted from tablets with ethanol containing (1,3-dimethyl-7-amil xanthine) (1 mg/ml) as the internal standard. A filtered aliquot was chromatographed with a 2% OV-17 steel column and the components were detected by a flame ionization detector. The method exhibited good linearity over the range 0.5-1.5 mg/ml (r=0.9997). Mean recovery of isbufylline added to tablet excipients was 99.07%. Mean assay results for 10 and 80 mg tablets were 95.72% and 96.73%, respectively. No degradation was detected during accelerated stability studies at elevated temperatures, confirming the excellent stability of this drug in tablets.

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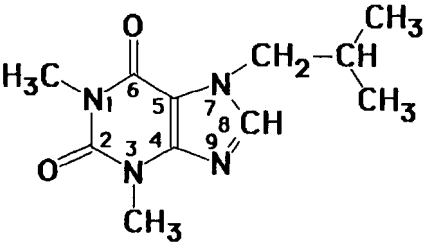
Introduction

Theophylline is still widely used in the treatment of chronic asthma (1). The major drawback with theophylline therapy is that the drug, at plasma concentrations above 20μg/ml, may produce toxic side-effects mainly due to CNS and cardiovascular stimulation (2,3). Therefore, there have been many attempts to make new and safer theophylline-like drugs lacking of central and cardiac stimulatory properties. In our research for new antiasthmatic agents, we have obtained promising activities with a new xanthine derivative: 1,3-dimethyl-7-isobutyl xanthine (Isbufylline I.N.N.; TE/06 laboratory name), a highly potent antibronchoconstrictor agent largely devoid of CNS stimulating effects (4). Isbufylline is currently undergoing phase I clinical evaluation and, therefore, previously described analytical procedures have been focused on the development of high performance liquid chromatographic (HPLC) methods for drug detection in biological matrices (5). In the present paper, a gas chromatographic for stability purpose analytical procedure is described for the assay of Isbufylline in tablets. This pharmaceutical form was investigated because the suggested route of drug administration is per oral. The chemical structure of Isbufylline is reported below.



EXPERIMENTAL SECTION

Apparatus

A Perkin Elmer (Norwalk, Connecticut, U.S.A.) 8500 Gas Chromatograph, equipped with a flame-ionization detector, and a 6-ft x 0.125-in steel column packed with 2% OV- 17, on Gas Chrom Q, 80-100 mesh, (Supelco, Inc., Bellefonte, PA), was used. All data were collected using a GP-100 Graphics Printer (Perkin Elmer). The samples (1μl) were injected into the column, with the injector port, detector block and column temperature set at 300, 300 and 225°C respectively. Helium was used

as the carrier gas at a flow rate of 25 ml/min. Detector response was optimized by setting the hydrogen and air pressure at12 and 22 psi, respectively.

Chemicals and reagents

Isbufylline and the internal standard (IS), 1,3-dimethyl-7-isoamil xanthine, were synthetized as previously described (4). Both compounds were at least 99% pure. Stock solutions of Isbufylline (5 mg/ml) and IS (5 mg/ml) were prepared in ethyl alcohol (Merck, Darmstadt, F.R.G.). Storage of these solutions at 4°C for at least three months, did not result in any detectable decomposition.

Tablet preparations

Two tablet dosage forms of Isbufylline, (10 and 80 mg), were prepared for stability studies. Tablet composition was the following:
Tablet (A)-Isbufylline 10 mg, microcrystalline cellulose (Avicel PH 102 F.M.C. Corporation, Philadelphia U.S.A.) 19.8 mg, unmilled dicalcium phosphate dihydrate (DI-TAB, Stauffer Chemicals, Westport U.S.A.) 29.7 mg and magnesium stearate (Carlo Erba, Milan Italy) 0.5 mg.
Tablet (B)-Isbufylline 80 mg , microcrystalline cellulose 63.4 mg, unmilled dicalcium phosphate dihydrate 95.1 mg, and magnesium stearate1.5 mg.
Tablets were compressed on a Ronchi CT 20 single punch tablet machine fitted with flat faced punches of 5 and 8.5 mm diameter for A and B tablets, respectively. Content of Isbufylline was evaluated by means of the GC analytical method described below. Physical tests (disintegration, dissolution and weight variation) were performed on each dosage form and were in agreement with U.S.P. XXI limits.

Sample preparation

Tablets from each preparation were weighed and ground to a fine powder. An accurately weighed quantity of powder, containing an equivalent of 25 mg of drug, was transferred into a 25 ml volumetric flask. A 5 ml aliquot of the IS solution was added, and the exact volume made up by adding ethyl alcohol. The mixture was stirred for 15 min at room temperature, and an aliquot filtered through a disposable Millipore filter Millex-SR, 0.2 µm (Molsheim, France). A 1 µl aliquot was then injected three times into the column, and the mean response was then used for the following quantitative analysis.

Table I **Temperature and time stability of isbufylline in tablets containing 10 mg of the drug.**

Temperature	Time	Appearence	Residual rate %[a]	Decomposition
	Initial	White	100	N.D.
	1 month	unchanged	100.1	N.D.
40°C	2 months	"	101.7	N.D.
	3 months	"	101.3	N.D.
	10 days	unchanged	100.0	N.D.
50°C	20 days	"	100.5	N.D.
	30 days	"	101.4	N.D.
	3 days	unchanged	98.9	N.D.
60°C	7 days	"	101.0	N.D.
	10 days	"	101.0	N.D.
25°C	1 year	unchanged	100.0	N.D.

N.D. not detectable

[a] each value represents the mean of three independent determinations and each determinations the mean of three single injections.

Calibration curve and samples analysis

Standard samples were prepared by adding 2.5, 3.75, 5, 6.25 or 7.5 ml of alcoholic solution containing 5 mg/ml of Isbufylline and 5 ml of alcoholic solution containing 5 mg/ml of IS in a 25 ml volumetric flask. The final volume was made up with the same solvent. The calibration curve was obtained by plotting the peak-area ratio of Isbufylline / IS against the concentration of Isbufylline in standard samples. Samples obtained from tablets were injected into the column and the concentration of Isbufylline was estimated from the regression curve by interpolation. Each day a new standard curve was generated.

Table II

Temperature and time stability of isbufylline in tablets containing 80 mg of the drug.

Temperature	Time	Appearence	Residual rate %[a]	Decomposition
	Initial	White	100.0	N.D.
	1 month	unchanged	101.1	N.D.
40°C	2 months	"	101.1	N.D.
	3 months	"	100.4	N.D.
	10 days	unchanged	100.0	N.D.
50°C	20 days	"	99.6	N.D.
	30 days	"	101.1	N.D.
	3 days	unchanged	98.6	N.D.
60°C	7 days	"	101.4	N.D.
	10 days	"	100.7	N.D.
25°C	1 year	unchanged	100.0	N.D.

N.D. not detectable

[a] each value rapresents the mean of three independent determinations and each determination was the mean of three single injections.

Procedure for assessing tablet stability

Accelerated stability studies at high temperatures (6) have been used as guides in the estimation of Isbufylline degradation. This procedure was based on the reproducibility of degradation rates function at various temperatures and extrapolation of the Arrhenius relation to obtain rate constants that would describe degradation rates at a room temperature of 25 °C. For this purpose an adequate number of tablets stored in glass containers were maintained at 40, 50

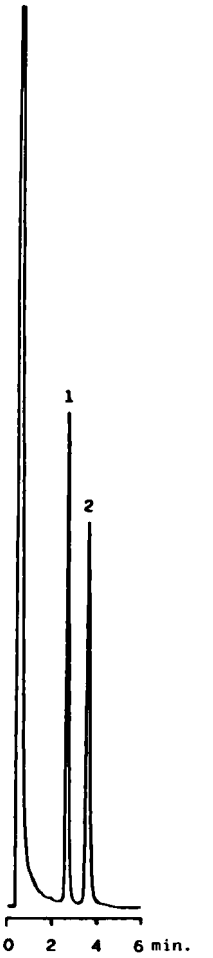


Fig. 1: Example of a GC chromatogram of a sample obtained from Isbufylline tablets additionate with I.S.. Peak 1=1 mg/ml Isbufylline, peak 2= 1 mg/ml I.S.

and 60 °C (± 1°C). Isbufylline contents in stored tablets were periodically assessed as reported in Table I and II, according to the GC method. Stability was also measured in tablets stored at room temperature (25°C) for one year.

RESULTS AND DISCUSSION

A typical chromatogram of a sample obtained from tablets is shown in figure 1. In these analytical conditions the retention times for Isbufylline and IS were 2.5 and

Table III

Gas chromatographic assay of isbufylline in tablets

Assay	10 mg Tablet		80 mg Tablet	
	mg/Tablet[a]	% of Label	mg/Tablet[a]	% of Label
1	9.55	95.5	77.28	96.6
2	9.56	95.6	76.80	96.0
3	9.34	93.4	76.96	96.2
4	9.45	94.5	77.92	97.4
5	9.46	94.6	77.36	96.7
6	9.81	98.1	77.80	96.4
7	9.65	96.5	76.40	95.5
8	9.66	96.6	79.20	99.0
9	9.66	96.6	77.80	97.24
10	9.58	95.8	77.08	96.35
Mean	9.57	95.72	77.46	96.73
S.D.	0.13	1.33	0.78	0.97
C.V.	0.14	1.39	1.01	1.00

[a] each value represents the mean of three independent determinations and each determinations rapresents the mean of three single injections.

3.4 min respectively, and the total analysis time was 6 min. It is noted that the excipients did not interfere with recovery and extraction of isbufylline from tablets.

To assess the percent recovery of Isbufylline and IS by means of the herein described procedure, tablets were prepared containing excipients and Isbufylline at three concentrations: 0.5, 1, and 1.5 mg/ml. After extraction, a comparison of the peak areas obtained with these extracted samples to those obtained by injection of standard solutions was made. The mean (± C.V.) recoveries of Isbufylline and IS were 99.07 ± 2.7% (n=6) and 98.03 ± 3.3% (n=6), respectively. The

intra-and inter-day precision of the assay was determined by performing six (intra) or twelve (inter) replicate analysis on 25 ml aliquots of ethyl alcohol solutions spiked with 25 mg of isbufylline and 25 mg of IS. The concentrations (± C.V.) of these samples were estimated from the regression curve and are 99.63 ± 1.23% and 99.86 ± 1.13% respectively. The mean (± C.V.) recovery of Isbufylline was 99.73 ± 0.20% (n=12). Good linearity was obtained for Isbufylline over the range 0.5-1.5 mg/ml and the correlation coefficient was 0.9997.

Samples from both tablet preparations were assessed for Isbufylline content by means of the proposed GC method. Table III summarizes the assay results and the pertinent statistical data. None of the samples showed interferences from the ecxipients.

The results concerning accelerated stability studies at high temperature of isbufylline tablets are shown in table I and II. No degradation was detected even at the highest temperature used, therefore, obviating any meaningful extrapolation by means of the Arrhenius relation. In addition no degradation was observed in tablets maintained at room temperature for one year. Present findings indicate that the proposed GC method is suitable for Isbufylline analysis in tablets. In addition Isbufylline in this pharmaceutical formulation is certainly stable for at least one year and, assuming from accelerated stability studies a degradation kinetic of first order (7), it might be safely stored at room temperature for a period longer than its commercial expiry date.

The method is simple, accurate, and stability-indicating, and, thus, suitable for routine quality control analysis, and for stability studies of Isbufylline containing tablet preparations.

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REFERENCES

1)M. Weinberger, ISI Atlas of Science Pharmacology, 53 (1981).

2) S.H. Snyder and P.J. Sklar, Psychiatr. Res.,18, 91 (1984).

3) C.G.A. Persson, J. Allergy Clin. Immunol.,78, 780 (1986).

4) M. Fedi and G. Bonacchi, U.S. Pat. 4544556 (1985)

5) O. Agostini, S. Manzini and Fedi M., J. Chromatogr. Biomed. Appl. 433, 288 (1988).

6) L. Braidotti and G.B. Crivellaro, Il Farmaco Ed. Pratica, 23, 509 (1968).

7) S.Vianello, N.Chenello and O. Chucconi , Il Farmaco Ed. Pratica, 28,65 (1976).