APPENDIX

OTHER RESEARCH PUBLICATIONS
Effect of some Hydrocolloids on the Crystal Size of Sulphaguanidine by Solvent Change Method

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The effect of hydrocolloids such as polyvinyl pyrrolidone (PVP), carboxymethyl cellulose (CMC), gelatin and sodium alginate (0.001 and 0.005%) on the particle size of sulphaguanidine produced by solvent change method of crystallization was studied. Hydrocolloids produced significant reduction in crystal size probably by the formation of a surface film around the crystal nuclei.

Among the different techniques of crystallization, solvent change method has been widely used. Several factors affecting the particle size of sulphadiazine produced by solvent change method under normal and turbulent agitational conditions have been reported. As the crystal size and shape are extremely important in pharmaceutical preparations, the consistency of crystal habit should be controlled. The influence of various added surface active agents to control the crystal size of sulphaguanidine and sodium urate has been reported. Polyelectrolytes like PVP and CMC have been reported to be useful for retarding crystal growth of sulphathiazole and barium sulphate. In vitro, precipitation of poorly soluble drugs, like phenyl hydantoin from non-aqueous vehicles in human plasma was recently reported. The effect of PVP, CMC, gelatin and sodium alginate on the crystal size of sulphaguanidine by solvent change method has been investigated.

EXPERIMENTAL

Materials: Sulphaguanidine I.P., dimethyl formamide (BDH), polyvinyl pyrrolidone 40,000 M.W. (BDH), carboxymethyl cellulose (BDH), gelatin (E. Merck) and sodium alginate (USSR).

Methods:

Crystallization of sulphaguanidine: Weighed quantities of sulphaguanidine to yield 10 and 20% w/v solutions were dissolved in 10 ml. of dimethyl formamide and then added slowly to 250 ml. of water. The contents were stirred at 52 rev./min. for 60 minutes and the separated crystals were filtered, washed and dried. Similarly, for overnight samples after the addition of the solution of sulphaguanidine in dimethyl formamide, the contents were stirred for one hour and kept for 24 hours and then filtered, washed and dried. To find out the effect of hydrocolloids, instead of pure water, 250 ml. of water containing 0.001 and 0.005% v/v of each of the hydrocolloids was used as non-solvent, and the crystallization of sulphaguanidine was carried out as described before. The particle size distribution of the crystals was determined microscopically. Not less than 300 crystals were measured. Infra red spectra of pure drug, hydrocolloids and nucleated drug in presence of hydrocolloids were taken by Pellet Bromide method using Perkin Elmer instrument.

X-ray analysis of crystal samples was made by using Philips x-ray unit and the photographs were taken by using a Debye-Scherrer Camera, using copper k radiation and exposed for 2 hours.

Other physical properties: Surface tensions of water samples containing hydrocolloids at the concentration of 0.001 and 0.005% were determined at room temperature by using Traube's Stalagmometer. Melting point of the
TABLE 1.—EFFECT OF HYDROCOLLOIDS ON THE SIZE OF THE CRYSTALS OF SULPHAGUANIDINE PRODUCED
BY SOLVENT CHANGE METHOD

<table>
<thead>
<tr>
<th>Hydrocolloids</th>
<th>10% drug</th>
<th>20% drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr**</td>
<td>24 hr</td>
</tr>
<tr>
<td>PVP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>10.78</td>
<td>9.045</td>
</tr>
<tr>
<td>(b)</td>
<td>9.89</td>
<td>8.803</td>
</tr>
<tr>
<td>CMC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>12.39</td>
<td>11.792</td>
</tr>
<tr>
<td>(b)</td>
<td>9.36</td>
<td>9.22</td>
</tr>
<tr>
<td>Gelatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>13.95</td>
<td>13.175</td>
</tr>
<tr>
<td>(b)</td>
<td>12.37</td>
<td>11.34</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>10.27</td>
<td>9.42</td>
</tr>
<tr>
<td>(b)</td>
<td>7.27</td>
<td>7.442</td>
</tr>
<tr>
<td>Control</td>
<td>53.53</td>
<td>43.175</td>
</tr>
</tbody>
</table>

Concentration of Hydrocolloid (a) 0.001% and (b) 0.005%.
* Mean of 300 crystals of sulphaguanidine, measured microscopically.
** Time intervals of crystallization.

The crystals were acicular in shape and were compared with the tabulated values.

RESULTS & DISCUSSION

The data obtained were subjected to a randomised block analysis of variance according to standard procedure. The crystals of sulphaguanidine obtained were acicular in shape and were assigned to size classes on the basis of

TABLE 2.—ANALYSIS OF VARIANCE SHOWING THE EFFECT OF SOLUTE CONCENTRATION AND TWO TIME
INTERVALS (ONE HOUR AND OVERNIGHT KEEPING) ON THE CRYSTAL SIZE OF SULPHAGUANIDINE PRODUCED
BY SOLVENT CHANGE METHOD AT TWO CONCENTRATION LEVELS OF ADDED HYDROCOLLOIDS

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>Sum of Squares</th>
<th>Degrees of freedom</th>
<th>Mean sum of squares</th>
<th>Variance ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Between the solute concentrations</td>
<td>34.598</td>
<td>19.666</td>
<td>1</td>
<td>34.598</td>
</tr>
<tr>
<td>Between time intervals</td>
<td>0.693</td>
<td>0.374</td>
<td>1</td>
<td>0.693</td>
</tr>
<tr>
<td>Interaction</td>
<td>1.264</td>
<td>0.184</td>
<td>1</td>
<td>1.264</td>
</tr>
<tr>
<td>Total of conditions of crystallization</td>
<td>36.555</td>
<td>20.225</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Between the Hydrocolloids</td>
<td>35.570</td>
<td>40.360</td>
<td>3</td>
<td>11.856</td>
</tr>
<tr>
<td>Error</td>
<td>3.963</td>
<td>1.145</td>
<td>9</td>
<td>0.4403</td>
</tr>
<tr>
<td>Total</td>
<td>76.088</td>
<td>61.730</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

a:—Hydrocolloid Conc. 0.001%, b:—Hydrocolloid Conc. 0.005%.
**:—Significant at 1% level.
their length. The mean length of crystals produced in all 36 series of crystallizations are shown in Table 1. The crystals formed appeared to be delicate due to their small diameter. At both the drug concentrations (10 and 20% w/v) the crystals of sulphaguanidine produced without any added hydrocolloids showed highly skewed distribution, whereas those formed in presence of hydrocolloids showed uniform growth. Further, the size of the crystals produced significantly differed among themselves between two concentrations of hydrocolloids (F being 26.91 and 105.6 at df 3/9 for 0.001 and 0.005% respectively as shown in Table 2). The crystals were identified as of sulphaguanidine by infra red spectrum. Fig. 1 shows a comparison of spectra of pure drug and drug nucleated in presence of 4 hydrocolloids. With the exception of some minor broadening of bands and a decrease in the absorption intensity, the spectra were identical. Among the 4 hydrocolloids, PVP showed less influence on the absorption intensity and CMC still less. New bands characteristic of the hydrocolloids were shown. It appears that hydrocolloids were preferentially adsorbed on the crystal nuclei and formed an interfacial barrier around the nuclei. The melting points for pure and crystal nucleated in presence of hydrocolloids showed good comparison.
The reported m.p. 193° was obtained for the pure sample and the nucleated samples melted at 190°. The difference of 3° as well as an observed discoloration of the nucleated drug samples upon melting may be attributed to traces of hydrocolloids adsorbed on the crystal or due to possible inclusion of trace amounts of poly electrolytes upon crystal formation. Surface tension values (dynes/cm²) for water containing hydrocolloids were as follows; PVP 67.71, 66.71; CMC 66.71, 66.61; gelatin 68.58, 67.85 and sodium alginate 66.49, 66.85. The X-ray diffractograms of pure drug as well as of the crystals nucleated in presence of hydrocolloids are shown in Fig. 2, which are identical in all respects, except for a few additional bands corresponding to the added hydrocolloids. Hence, no polymorphism but only change in the crystal size seems to take place as evidenced by the increase in the width of the bands. In the case of crystals nucleated in presence of gelatin and sodium alginate, the difference in the pattern can be attributed to the influence of patterns of sodium alginate and gelatin, which are having definite x-ray patterns of their own. The drug concentration in dimethyl formamide was found to affect significantly the size of crystals. At both levels of concentrations of hydrocolloids the difference in crystal size between one hour and 24 hours samples was not significant as shown in Table 2. The effect of hydrocolloids in reducing the crystal size, therefore, cannot be attributed to any single factor in solvent change crystallization process. The charges of individual hydrocolloids also probably play a role in their adsorption on the crystal surface.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. A. Affonso, Principal, Goa College of Pharmacy, for providing facilities for the work and to Shri G. R. Kamat, Engineer in charge, Foundry, and to Shri N. A. Narasimhan, Head Spectroscopy division, Bhaba Atomic Research Centre, Bombay, for their co-operation in undertaking X-ray and I.R. Spectral analysis of the samples.

REFERENCES


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Antimicrobial Activity of Metallic Salts of Arabic Acid

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ABSTRACT
The use of mercury, copper, iron and lead has been mentioned in Ayurveda. The inorganic salts of the metals were toxic and caused irritation. They precipitated proteins due to high concentration of metallic ions. They could not be used on the skin over a long period of time. Hence attempts were made to overcome the above disadvantages by incorporating the metals like mercury, silver and copper in complex organic acid like arabic acid prepared from gum acacia. These metallic salts of arabic acid were then screened against different bacteria and fungi, and they were found to possess both antibacterial and antifungal properties.

INTRODUCTION
Gum arabic as found in nature is a mixture of calcium, magnesium and potassium salts of arabic acid. It contains 70.40 per cent of arabic acid and 17.6 per cent of water. There are several methods for the preparation of arabic acid and salts of arabic acid. The present work is mainly focussed on the screening of the anti-microbial activity of metallic salts of arabic acid as these salts have not been studied so far for their antimicrobial activity.

METHOD
Preparation of arabic acid
A 10 per cent gum acacia solution was prepared in 0.1N hydrochloric acid by boiling on water bath for one hour. The solution was then filtered and dialysed. It was found that no calcium, magnesium, and potassium ions could be detected after 48 hrs. dialysis, while gum acacia originally contained 0.058 per cent potassium, 0.8076 per cent calcium and 0.7409 per cent magnesium. Arabic acid was then precipitated by adding acetone to the above dialysed gum acacia solution, filtered and dried in vacuum desiccator. The percentage yield was 68 per cent.

Preparation of Metallic Salts of Arabic Acids
Arabic acid was dissolved in the required amount of 0.1N sodium hydroxide solution and dialysed for 12 hrs. to free from sodium ions. This sodium arabate solution was then complexed with equivalent amount of mercury nitrate solution and the mixture was maintained at 50°C for 3 hrs., filtered and then dialysed to free from mercury ions. The dialysed mercury arabate solution was then precipitated, filtered and dried as above. In the same manner, silver arabate and copper arabate were prepared by complexing with silver nitrate and copper nitrate solution respectively. Copper arabate and silver arabate were estimated for their copper and silver content by I.P. method and mercury arabate was estimated for its mercury content by B.P.C. 1963 method. It was found to contain 0.4133 g per cent of copper, 0.6905 g per cent of silver and 0.2507 g per cent of mercury ion.

Preparation of Test Samples
A 1 per cent of the metal arabates was prepared in distilled water aseptically and evaluated for antimicrobial activity by cylinder plate method of florey and chain.
Standards

Penicillin, sodium I.P. (2.5 units/ml.) and salicylic acid 1 per cent were used as standard substances for antibacterial and antifungal activities respectively.

The oxoid Nutrient agar and Sabourand's medium were used for growing bacteria and fungi respectively. The experiments were conducted in Petri dishes (150 mm. X 20 mm.) containing 50 ml. of nutrient medium. An actively growing 24 hrs. old culture of the test organisms was used as inoculum. Short, open ended steel cylinders (8 mm. O.D.) were placed on the surface of medium and slightly pressed in the medium. They were then filled with required quantity of metal arabate solution. The plates were then refrigerated for 2 hrs. to allow the diffusion of metal arabate solution and then incubated for 24 hrs. at 37°C. The diameter of zone of inhibition was then measured. Control and standard experiments were also run simultaneously. Observations are recorded in Table I and Table II.

| TABLE — I | ANTIBACTERIAL ACTIVITY OF METAL ARABATES COMPARED WITH PENICILLIN SODIUM I.P. |
|-----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|------------------|------------------|
| Organism        | Distilled water (Control)       | Mercury arabate 1% | Silver arabate 1% | Copper arabate sodium I.P. 1% | Penicillin 2.5 units/ml. |
| Escherichia coli| —                               | 10              | —                | —                | —                | —                |
| Bacillus subtilis| —                               | 14             | 13               | 25               | —                |
| " anthraces     | —                               | 14             | 12               | 10               | 29               |
| " pneumus       | —                               | 13             | 10               | 8                | 18               |
| Salmonella typhosa| —                              | 13             | 10               | 7                | —                |
| Straphylococcus aureus| —                              | 12             | 9                | 7                | 18               |
| Micrococcus pyogenes| —                              | 16             | 14               | 11               | 22               |
| Proteus vulgaris| —                               | 14             | 10               | —                | —                |

| TABLE — II | ANTIFUNGAL ACTIVITY OF METAL ARABATES COMPARED WITH SALICYLIC ACID |
|-----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|------------------|------------------|
| Organism        | Distilled Water                 | Mercury arabate 1% | Silver arabate 1% | Copper arabate 1% | Salicylic 1% |
| Aspergillus niger| —                               | 20             | 18               | 16               | 34               |
| " flavous       | —                               | 19             | 16               | 14               | 34               |
| Trichophyton equinopus| —                               | 16             | 12               | 9                | 37               |
| Fusarium oxysporum| —                               | 10             | —                | —                | —                |
| Cryptococcus neoformans| —                               | 18             | 16               | 12               | 27               |

* Including the diameter of the cylinder.

** Mean of three readings.
DISCUSSION

In vitro, antibacterial and antifungal activities, of some metal arabates prepared from arabic acid of gum acacia were determined using cylinder plate method against some bacteria and fungi. The maximum antimicrobial activity was observed against Micrococcus pyogenes and Aspergillus niger as compared with Penicillin and Salicylic acid. From Table I and Table II it is evident that the metallic salts prepared from arabic acid possess potent antibacterial and antifungal activities.

ACKNOWLEDGEMENTS

The author is indebted to Dr. A. Affonso, Principal, Goa College of Pharmacy, Panaji for his valuable suggestions in the work.

REFERENCES

In Vitro, Comparison of Acid consuming capacity, Specific Rate constant and Buffering capacity of Commercial Antacid Tablets.

R. V. GAITONDE
& B. S. NATH
Goa College of Pharmacy,
Panaji-Goa.

ABSTRACT

Eleven commercial antacid tablets containing various amounts of different antacid ingredients were tested in Vitro for acid consuming capacity, specific rate constant and buffering capacity in maintaining an elevated PH which varied over a wide range among the samples tested.

INTRODUCTION

Gastric antacids are drugs which are used for the treatment of hyperchlorhydria and peptic ulcer. The properties of the antacids, especially alumina gel, have been reported, to be effected during preparation by several factors. The effectiveness of an antacid formulation depends not only on its ability to neutralise gastric hydrochloric acid immediately after dosing but also on its ability to maintain intragastric pH at an elevated level favourable to ulcer healing and above that optimal for pepsin activity. The additives added during formulation of antacid tablets may have some effect on the acid properties. Effect of some granulating agents on antacid properties of dried alumium hydroxide gel has been reported. The marketed antacid preparations with varied amounts of different ingredients, prepared with different binding agents were therefore tested for their antacid properties.

EXPERIMENTAL

In order to carry out the tests, code letters were assigned to each of the eleven commercial antacid tested. Samples from different lots of each antacid tablet were powdered and a weighed quantity of powder was taken for all the tests.

METHOD

Acid consuming capacity was determined as per I.P. method and was calculated in terms of volume of 1 N acid consumed per gram of sample using the formula reported in our previous article. Buffering capacity was determined as per the method recommended by Hobert et al, using PR 9405 L PH meter of Philips make and was calculated by the method reported in our previous article.

Specific rate constant was determined and calculated by the method reported in our previous article.

RESULTS AND DISCUSSION

The wide variation in acid consuming capacity among the antacid formulations can be explained by the fact that the formulations are combinations of different antacid ingredients having different acid consuming potencies or contain the same ingredients in different ratios or chemical forms. Formulation F had the highest acid consuming capacity and formulations D & H had the longest duration of action. However, the correlation between acid consuming capacity and buffering capacity was not close with most of the other formulations. Of course, these in Vitro, buffering capacity data cannot be directly applied to as in Viva system, because while simulated gastric fluid was added to the in-vitro system, at a constant rate, the stomach secretes gastric acid at a variable rate. The specific rate constant values inversely indicate the rate at which the acid was neutralised by the sample. Higher the values of specific rate constant, the lesser would be the acid neutralised by samples in that time interval. Lastly, it can be concluded that there is a considerable difference among the commercial antacid preparations tested in
### Table 1

**ACID CONSUMING CAPACITY AND SPECIFIC RATE CONSTANT OF ELEVEN COMMERCIAL ANTACID TABLETS**

O.1 N HCl. Consumed by 1 gm. of sample in given replicate determination.

<table>
<thead>
<tr>
<th>Sample symbol</th>
<th>Acid consuming capacity in C.C.</th>
<th>Specific rate constant K = Min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>182.8</td>
<td>186.8</td>
</tr>
<tr>
<td>B</td>
<td>87.6</td>
<td>84.4</td>
</tr>
<tr>
<td>C</td>
<td>127.2</td>
<td>128.4</td>
</tr>
<tr>
<td>D</td>
<td>164.4</td>
<td>163.6</td>
</tr>
<tr>
<td>E</td>
<td>94.8</td>
<td>94.0</td>
</tr>
<tr>
<td>F</td>
<td>190.8</td>
<td>192.0</td>
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<td>G</td>
<td>87.2</td>
<td>85.2</td>
</tr>
<tr>
<td>H</td>
<td>184.0</td>
<td>184.0</td>
</tr>
<tr>
<td>I</td>
<td>146.0</td>
<td>143.6</td>
</tr>
<tr>
<td>J</td>
<td>150.0</td>
<td>150.0</td>
</tr>
<tr>
<td>K</td>
<td>91.2</td>
<td>89.2</td>
</tr>
</tbody>
</table>

**Table 2**

**BUFFERING CAPACITY OF ELEVEN COMMERCIAL ANTACID TABLETS**

<table>
<thead>
<tr>
<th>Sample symbol</th>
<th>pH at time intervals of minutes.</th>
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</thead>
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<tr>
<td></td>
<td>0-5</td>
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<tr>
<td>A</td>
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<tr>
<td>B</td>
<td>1.65</td>
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<tr>
<td>C</td>
<td>1.5</td>
</tr>
<tr>
<td>D</td>
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<td>E</td>
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<td>F</td>
<td>1.8</td>
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<tr>
<td>G</td>
<td>1.6</td>
</tr>
<tr>
<td>H</td>
<td>1.6</td>
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<tr>
<td>I</td>
<td>1.65</td>
</tr>
<tr>
<td>J</td>
<td>1.9</td>
</tr>
<tr>
<td>K</td>
<td>1.8</td>
</tr>
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</table>

Acknowledgements

Authors' wish to thank Prof. K. K. Kapur, Ag. Principal, Goa College of Pharmacy, for his kind interest shown in the work and Dr. M. Zingde, Senior Scientific Officer, N.I.O., Panaji-Goa for his technical assistance.

References

PREPARATION AND FACTORS INFLUENCING THE DIPHENHYDRMINE HYDROCHLORIDE CARRAGEENAN (TYPE I) COMPLEX

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At 1:1 stoichiometric ratio carrageenan type I was found to form a free flowing complex at room temperature with diphenhydramine hydrochloride when added as dry powder or in solution. pH of 5.6 was found to be optimum for better yield and drug payload when pH effects were investigated. The complex was found to dissociate the drug within a period of three hours in simulated gastric, pancreatic fluids and in water. The release data when plotted was found to obey Higuchi's square root law.

In addition to active drug formulations contain other ingredients hitherto considered to be inert. Several reports have shown that added excipients will interact in one way or the other with the active ingredients and effect the bioavailability. Natural gums and other cellulose derivatives were found to interact from highly insoluble complexes with antihistamines and other drugs with little product applications. The interacting tendencies of carrageenan type I (80% kappa and 20% lambda varieties with a view to isolate a soluble complex of diphenhydramine hydrochloride was studied under a variety of experimental conditions and recovered as dry powder and further characterised for its flow behavior. The in vitro drug release was studied in water, simulated gastric and pancreatic fluids.

EXPERIMENTAL

Materials:—Carrageenan type I—Sigma Chemicals (7-SA), diphenhydramine hydrochloride—I.P., Bromothymol blue—E. Merck, citric acid—BDH, Sodium phosphate dibasic—Sarabhai, chloroform—I.P., Hydroch gloic acid—E. Merck were used.

Preparation and isolation of Carrageenan diphenhydramine hydrochloride complex: A quantity of 25 ml uniformly dispersed gum solution (2%w/v) in a 100 ml beaker to which a quantity of 25 ml of the drug solution (2%w/v) in distilled water, was added with stirring at a speed of 100-150 rpm at room temperature (28°C). The precipitate which was formed immediately was allowed to stand for a period of 10 minutes and filtered through whatman no. 1 filter paper. The filtrate was collected to last drop in which the unreacted drug content was determined colorimetrically by adopting acid-dye method. The precipitate was dried as 60°C for 3 hours and pulvemised in a glass mortar for further characterisation.

The complex was also prepared by adding the drug in dry form to a quantity of 25 ml of 2% gum solution.

Effect of pH on the yield and drug payload of the complex: was determined by carrying out the reaction in McIlvaine buffers of various pH values such as 2.2, 3.6, 4.6, 5.6, 6.6 and 7.6 for dissolving the gum. In these experiments the drug was added in dry form.

Physico-chemical properties: Size and size distribution of the complex was determined by sieve analysis.

Table I

Effect of mode of addition of Diphenhydramine Hydrochloride on the extent of binding to Carrageenan

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drug free mgs</th>
<th>Drug in Complex mgs</th>
<th>Dried recovered Complex mgs</th>
<th>Drug bound mg/gm</th>
<th>Drug free mgs</th>
<th>Drug in Complex mgs</th>
<th>Recovered Complex mgs</th>
<th>Drug bound mg/gm</th>
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<tbody>
<tr>
<td>1</td>
<td>302.94</td>
<td>197.06</td>
<td>425.313</td>
<td>463.3294</td>
<td>245.548</td>
<td>254.452</td>
<td>487.578</td>
<td>521.8693</td>
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<tr>
<td>2</td>
<td>302.94</td>
<td>197.06</td>
<td>425.701</td>
<td>462.9071</td>
<td>249.668</td>
<td>250.323</td>
<td>495.656</td>
<td>505.0519</td>
</tr>
<tr>
<td>3</td>
<td>302.72</td>
<td>197.23</td>
<td>428.062</td>
<td>456.1956</td>
<td>249.668</td>
<td>250.332</td>
<td>491.650</td>
<td>509.1671</td>
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<tr>
<td>4</td>
<td>304.14</td>
<td>195.86</td>
<td>425.808</td>
<td>459.9726</td>
<td>251.568</td>
<td>248.432</td>
<td>494.676</td>
<td>492.1039</td>
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<td>5</td>
<td>302.94</td>
<td>197.06</td>
<td>420.362</td>
<td>468.7864</td>
<td>249.668</td>
<td>250.332</td>
<td>485.220</td>
<td>515.9144</td>
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<tr>
<td>Mean</td>
<td>303.136</td>
<td>196.864</td>
<td>425.0492</td>
<td>462.2312</td>
<td>249.224</td>
<td>250.776</td>
<td>490.946</td>
<td>508.8213</td>
</tr>
</tbody>
</table>

Drug added is 500 mg and Carrageenan taken in 500 mg.
Table II
The effect of solution pH on the extent of binding of Diphenhydramine Hydrochloride to Carrageenan

<table>
<thead>
<tr>
<th>pH</th>
<th>Drug free mgs</th>
<th>Drug in Complex mgs</th>
<th>Weight of Complex mgs</th>
<th>Drug bound mg/gm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>284.496</td>
<td>225.504</td>
<td>454.944</td>
<td>473.694</td>
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<td>3.6</td>
<td>270.506</td>
<td>229.494</td>
<td>476.612</td>
<td>488.897</td>
</tr>
<tr>
<td>4.6</td>
<td>264.668</td>
<td>235.332</td>
<td>474.234</td>
<td>496.236</td>
</tr>
<tr>
<td>5.6</td>
<td>255.328</td>
<td>244.672</td>
<td>482.126</td>
<td>507.486</td>
</tr>
<tr>
<td>6.6</td>
<td>283.784</td>
<td>216.216</td>
<td>450.412</td>
<td>483.040</td>
</tr>
<tr>
<td>7.6</td>
<td>294.88</td>
<td>205.12</td>
<td>447.828</td>
<td>458.623</td>
</tr>
</tbody>
</table>

Drug added is 500 mg. Carrageenan taken 500 mg in 25 ml of buffer solutions. Each reading is mean of 5 replicates.

using "Endicot" seives 10, 20, 44, 60, 100, 120 and 170 with shaking time of 30 minutes using a mechanical shaker. True density was determined using a specific gravity bottle with toluene as immersion fluid at 28°C. Bulk density was determined by adopting the reported method of Butler and Ramsey. Tap density was found by tapping a weighed amount of the complex in a 10 ml measuring cylinder using a mechanical tapping device (120 taps/min). The per cent bed porosity values were computed from true and bulk densities. The angle of repose (Theta) was determined by using Pilpel cylinder method. Moisture content was determined by using infra red moisture balance. Rate of packing from loose to tight packing was determined as per reported method. Powdered X-ray diffractograms were taken using PW 1010 Philips X-ray diffractometer with CuK radiation and Nickel filter at scanning speed of 2θ/min and scanning range (2θ) of 6-70°. Infra red spectra was taken by pellet bromide method using Perkin-Elmer spectrometer.

In vitro dissolution of the complex: was determined in water, simulated gastric and pancreatic fluids by following beaker and stirrer method of Levy and Hays by taking a quantity of 50 mgs accurately weighed complex tied in a muslin cloth bag and 250 ml of respective dissolution fluids. A 10 ml samples were withdrawn at each 30 minutes interval upto a period of 3 hours and immediately replaced by some quantity of fresh dissolution fluids. The drug released at each interval of time

Table III
Analysis of variance showing the effect of solution pH on the extent of binding and yield of diphenhydramine hydrochloride to Carrageenan

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Sum of Squares</th>
<th>Degrees of freedom (Di)</th>
<th>Mean sum of Squares</th>
<th>Variance ratio</th>
<th>Tabulated value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
</tr>
<tr>
<td>Between pH values</td>
<td>6298.8748</td>
<td>5562.2825</td>
<td>1259.7749</td>
<td>1112.45</td>
<td>7.7776</td>
</tr>
<tr>
<td>Between replicate</td>
<td>2432.0499</td>
<td>644.7929</td>
<td>358.0225</td>
<td>161.198</td>
<td>2.2101</td>
</tr>
<tr>
<td>Error</td>
<td>3239.783</td>
<td>1080.4283</td>
<td>161.989</td>
<td>54.0219</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10970.707</td>
<td>6642.7198</td>
<td>29</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

a= drug bound  
b= yield  
Significance at 5% level of Probability P=0.5 at Di 5 and 20
Table IV

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>Water C.R.</th>
<th>Water %C.R.</th>
<th>Simulated gastric fluid PH 1.1 C.R.</th>
<th>Simulated gastric fluid %C.R.</th>
<th>Simulated Intestinal fluid pH 7.6 C.R.</th>
<th>Simulated Intestinal fluid %C.R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>6.68</td>
<td>26.70</td>
<td>15.43</td>
<td>61.67</td>
<td>7.31</td>
<td>29.22</td>
</tr>
<tr>
<td>60</td>
<td>9.47</td>
<td>37.85</td>
<td>17.76</td>
<td>70.98</td>
<td>9.14</td>
<td>36.53</td>
</tr>
<tr>
<td>90</td>
<td>11.81</td>
<td>47.20</td>
<td>28.49</td>
<td>73.90</td>
<td>10.73</td>
<td>42.89</td>
</tr>
<tr>
<td>120</td>
<td>13.57</td>
<td>45.24</td>
<td>19.49</td>
<td>77.90</td>
<td>12.18</td>
<td>48.68</td>
</tr>
<tr>
<td>150</td>
<td>14.98</td>
<td>59.87</td>
<td>20.42</td>
<td>81.61</td>
<td>13.44</td>
<td>53.72</td>
</tr>
<tr>
<td>180</td>
<td>15.40</td>
<td>61.55</td>
<td>20.98</td>
<td>83.83</td>
<td>14.74</td>
<td>58.91</td>
</tr>
</tbody>
</table>

Each 50 mg of complex represents 25.02 mg of active drug.

C.R. = Cumulative release.

was determined colorimetrically.

RESULTS AND DISCUSSION

The effect of mode of addition of diphenhydramine hydrochloride to carrageenan solution on the yield and drug payload of the resulting complex was investigated and the results are shown in table 1. The yield and payload in mg and mgs/gm or dry complex in direct and indirect method of drug addition were 490.96, 508.82 and 425.05, 462.24 respectively. So, the results showed that the direct addition of drug to gum solution favoured better yield and drug payload.

Fig. I

The results shown in table II revealed that at pH 5.6 maximum amount of yield with good drug payload was obtained in comparison to any other pH value studied. The reason may be attributed to the least solubility of the complex at that pH. Perhaps pH 5.6 may be considered as the isoelectric point of the complex. Precisely to know the effect of different pH values on the complex formation, analysis of variance was carried out on both the values of yield and drug payload at each pH value and shown in table III. Variance between the different pH values was found to be significant both in the case of yield as well as drug payload as shown by F values obtained which were 7.7769 and 20.59 respectively. These values were far greater than the tabulated value of 3.1 to be significant at P<0.05 with df 5 and 20.

Fig. II

RATE OF PACKING OF DIPHENHYDRAMINE HYDROCHLORIDE CARRAGEENAN COMPLEX PARTICLES

THE EASTERN PHARMACIST—AUGUST, 1984
APPLICATION OF SPECTROPHOTOMETRY FOR ESTIMATION OF TRIAMCINOLONE ACETONIDE IN ITS DOSAGE FORMS

Triamcinolone acetonide (TCA) is an antiinflammatory gluocorticoid, chemically known as 9, fluoro-11, 12-dihydroxy-16, 17-1-methylidene bis (oxy)pregna-1, 4-diene-3, 20-dione. It is official in B.P.'8U and U.S.P. XX/N.F. XVI.

Official methods of estimation are U.V. spectrophotometry for pure drug\(^1\) and HPLC for pure drug and formulations\(^2\). Other reported procedures include Polarography\(^3\), qualitative paper chromatography\(^4\), column chromatography\(^5\), U.V absorptionmetry for estimation in body fluids\(^6,7\), HPLC\(^8\). Gas chromatography\(^9\) and colorimetry using tetrazolium blue\(^10,12\) and by reaction with isoniazide\(^13\).

The present communication describes a single colorimetric procedure based on quantitative colorimetric reaction of TCA with 4-amino antipyrine. The chromogenic species resulted is quantified at its absorbance maxima of 385 nm. The validity of Beer's law was ascertained by linearity of the calibration curve over the concentration range of 5 - 50 mcg/ml.

Instruments; Shimadzu UV 240 U.V./Visible recording spectrophotometer, Japan.

1. Solution of 4-amino antipyrine - 0.5% W/V is methanol containing 1 ml of hydrochloric acid per 100 ml.

Preparation of standard drug solution:—

The authenticity of a reference sample of TCA was ascertained by B.P. method. 10 mg of the drug was dissolved in methanol so as to obtain 100 ml solution containing 100 mcg/ml of TCA.

Preparation of sample solution:—

1. Ointments and creams:—

Accurately weighed sample containing 10 mg of TCA was transferred to a separating funnel containing 100 ml of cyclohexane and 50 ml of methanol and shaken for 15 minutes. After separation of two layers the bottom layer was treated with 250 ml of water containing about 5 g of sodium chloride and the combined extract was evaporated to dryness. The residue was transferred to 100 ml volumetric flask by dissolving in methanol and same solvent was used to make up the volume.

2. Sterile suspension:—

Contents of five vials were thoroughly mixed and quantity of suspension equivalent to 10 mg of TCA was transferred to a 100 ml volumetric flask. About 60 ml of methanol was added to the flask and shaken vigorously for 30 minutes. The volume was adjusted with methanol and the solution was filtered through a filter paper. The filtrate after rejecting first 30 ml was used for analysis.

3. Tablets containing Triamcinolone acetone:—

20 tablets were accurately weighed and finely pulverised. Quantity of powder containing 9.08 mg of triamcinolone (equivalent to 10 mg of TCA) was successively extracted with 4 X 20 ml portions of methanol. Each extract was filtered through a filtered paper into a 100 ml volumetric flask. Additional 10 ml of methanol were passed through the filter and the volume was made up with the same solvent.

Method of analysis:—

1,2,3, and 4 ml aliquots from the sample solutions taken in a series of 10 ml volumetric flasks
were treated with 2 ml of solution of 4-amino antipyrene and the flasks were immersed for fifteen minutes in a waterbath maintained at 50° ±20 °C. After cooling the volume was made up with methanol and the absorbances were recorded at 385 nm against a reagent blank. Various volumes of the standard drug solution were treated in an identical manner and a standard curve was obtained by plotting the absorbances against the known concentrations. The drug content of the sample were then established from the standard curve. The results were statistically evaluated and recovery experiments were carried out. The results are presented in Table 1.

Optimisation of the reaction conditions was effected through various preliminary experiments. It is evident from the results of the statistical analysis and the recovery studies that the suggested method is reproducible and reliable for the quantitation of TCA in its formulations. It is simple, sensitive and requires less investment as compared to the U.S.P. procedure. No interference was observed from neomycin, nystatin and gramicidin. A tablet containing triamcinolone base was successfully analysed, the drug content being computed from the molecular equivalents. The authors expresses their sincere thanks to Principal of Goa College of Pharmacy for providing necessary research facilities; Prof. O. S. Kamalapurkar for his helpful guidance and Cynamid (I) Ltd. for a gift of authentic drug sample.

TABLE 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labelled claim per 1ml/1g/tablet mg</th>
<th>Amount of drug* found by proposed method mg</th>
<th>Percentage recovery</th>
<th>Standard deviation</th>
<th>C.V. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile suspension</td>
<td>10</td>
<td>10.229</td>
<td>100.55</td>
<td>±±0.112</td>
<td>1.0990</td>
</tr>
<tr>
<td>Ointments</td>
<td>1</td>
<td>0.966</td>
<td>99.39</td>
<td>±±0.0096</td>
<td>0.9903</td>
</tr>
<tr>
<td>O1</td>
<td>1</td>
<td>0.972</td>
<td>100.66</td>
<td>±±0.0083</td>
<td>0.8484</td>
</tr>
<tr>
<td>Cream C</td>
<td>1</td>
<td>1.003</td>
<td>100.55</td>
<td>±±0.0052</td>
<td>0.5199</td>
</tr>
<tr>
<td>Table T, **</td>
<td>4</td>
<td>3.906</td>
<td>97.59</td>
<td>±±0.025</td>
<td>0.636</td>
</tr>
</tbody>
</table>

* Each result is a mean of four replicates.

** Content of triamcinolone base.
REFERENCES


5. Poet, P; Squibb, Personal communication, through Anal. Prof. vol. I (1972).


Pharmaceutical Research Laboratory
Goa College of Pharmacy,
Panaji, Goa, 403001
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SHORT NOTE

ANALYSIS OF A DRUG PREPARATION CONTAINING NOSCAPINE, EPHEDRINE HYDROCHLORIDE AND CHLORPHENIRAMINE MALEATE BY THIN LAYER CHROMATOGRAPHY.

Pharmaceutical preparations are the complex mixtures of several drugs each having its own effect. As far as the analysis of such preparations is concerned, interference of one drug into another is observed usually. Also the official methods which are adopted for it are lengthy, tedious and often does not give reproducible results. Therefore the aim of this part of present endeavour is to separate the components of complex drug preparation on a single chromatoplate and evaluate the components by spectrophotometric method. This chromatographic method is sensitive, accurate, fast, versatile, economical and reproducible.

Shimadzu — U V 240 / visible recording spectrophotometer.

As all the three drugs absorb light in uv range, they were quantitated by ultraviolet absorption spectrophotometry.

Noscapine in 95% ethanol exhibits an absorption maxima at 291 nm (E 1% 1 cm 92) while ephedrine hydrochloride in 0.1N hydrochloric acid exhibits an absorption maxima at 253 nm (E 1% 1 cm 8) and chlorpheniramine maleate in 0.1 N sulphuric acid exhibits an absorption maxima at 265 nm (E 1% 1 cm 212).

The label claims per capsule are

Noscapine 8 mg
Ephedrine hydrochloride 8 mg
Chlorpheniramine maleate 2 mg

1) 8 mg of noscapine was dissolved in 100 ml of ethanol.
2) 8 mg of ephedrine hydrochloride was dissolved in 100 ml of ethanol.

3) 2 mg of chlorpheniramine maleate was dissolved in 100 ml of ethanol.

20 capsules were weighed and their contents were removed. The empty shells were weighed. The content was mixed. From this, content equivalent to one capsule was transferred to a 250 ml beaker. 70 ml of ethanol was added to this and shaken for 5 minutes. The solution was filtered through whatman no. 42 paper in a 100 ml volumetric flask. The residue was washed with 5 ml each of ethanol 3-4 times and the volume was made upto 100 ml with ethanol.

8 mg of noscapine, 8 mg of ephedrine hydrochloride and 2 mg of chlorpheniramine maleate were weighed accurately and dissolved in 100 ml of ethanol.

The sample of noscapine was checked for its purity by B.P. specifications while that of ephedrine hydrochloride and chlorpheniramine maleate by I.P. specifications.

The glass plates were coated with silica gel G.* The dried plate was divided into three parts, the central one kept for blank while the remaining two for test and standard solutions each. Likewise three chromatoplates were prepared. 0.25, 0.5 and 0.75 ml of each of standard and test solution was streaked on the respective section of the plates. The plates were developed using the solvent system chloroform : benzene : n-butanol : methanol — (30 : 12 : 6 : 4). The drugs were separated at following Rf values —

Noscapine 0.95
Ephedrine hydrochloride 0.10
Chlorpheniramine maleate 0.80

INDIAN DRUGS, 1986, 23 (10)
The resulting band each of the above drug was scrapped out from the plate and treated in following manner —

a) The scrappings of the band corresponding to noscapine were transferred to a 250 ml beaker. 70 ml of 95% ethanol was added to it and stirred. The solution was filtered in a 100 ml volumetric flask. The residue was washed with 5 ml each of 95% ethanol 3-4 times. The washings were added to filtrate and volume was adjusted to 100 ml with 95% ethanol. This procedure was done with standard, test and also the corresponding portion of blank section.

b) The scrappings of the band corresponding to ephedrine hydrochloride were transferred to 250 ml beaker. 70 ml of 0.1 N hydrochloric acid was added to it and stirred. The solution was filtered in a 100 ml volumetric flask. The residue was washed with 5 ml each of 0.1 N hydrochloric acid 3-4 times. The washings were added to filtrate and volume was adjusted to 100 ml with 0.1 N hydrochloric acid. This procedure was done with standard, test and also the corresponding portion of blank section.

c) The scrappings of the band corresponding to chlorpheniramine maleate were transferred to a 250 ml beaker. 70 ml of 0.1 N sulphuric acid was added to it and stirred. The solution was filtered in a 100 ml volumetric flask. The residue was washed with 5 ml each of 0.1 N sulphuric acid 3-4 times. The washings were added to filtrate and volume was adjusted to 100 ml with 0.1 N sulphuric acid. This procedure was done with standard, test and also the corresponding portion of blank section.

To the content of 5 capsules, 8 mg of noscapine, 8 mg of ephedrine hydrochloride and 2 mg of chlorpheniramine maleate were added. From this admixture the quantity of content equivalent to 8 mg of noscapine was taken and analysed by the proposed method. The percentage recovery of the active ingredients were computed from the results obtained. Further statistical evaluation indicated the precision of the proposed method.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Labelled content per capsule in mg</th>
<th>Content per capsule by proposed method in mg</th>
<th>Amount of drug recovered in mg</th>
<th>% Recovery</th>
<th>Standard deviation</th>
<th>Coefficient of variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noscapine</td>
<td>8</td>
<td>8.0502</td>
<td>8.1960</td>
<td>102.45</td>
<td>±0.05339</td>
<td>0.66513</td>
</tr>
<tr>
<td>Ephedrine hydrochloride</td>
<td>8</td>
<td>7.9788</td>
<td>8.1018</td>
<td>101.27</td>
<td>±0.22141</td>
<td>2.78641</td>
</tr>
<tr>
<td>Chlorpheniramine maleate</td>
<td>2</td>
<td>2.0101</td>
<td>1.9899</td>
<td>99.49</td>
<td>±0.05873</td>
<td>2.88303</td>
</tr>
</tbody>
</table>

The authors are very much thankful to Prof. J. Emanuel, Acting Principal, Goa College of Pharmacy for providing necessary facilities for this research work.

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Phytochemistry Research Labs.
Goa College of Pharmacy, Panaji, Goa-403 001.
23 October 1985

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SHORT RESEARCH COMMUNICATION

TLC - SPECTROPHOTOMETRIC ANALYSIS OF STRYCHNINE AND BRUCINE FROM THE AYURVEDIC PILLS OF NUX VOMICA

R. V. GAITONDE and SANJAY JOSHI
Phytochemistry Lab., Goa College of Pharmacy, Panaji-Goa-403 001, India

ABSTRACT: Ayurvedic preparations claim on their label only the quantity of crude drugs and not the quantity of active ingredients present therein. So work was taken up to find the percentage of strychnine and brucine from Ayurvedic pills of Nux vomica powder by TLC spectrophotometric analysis, which study has not been reported earlier. However, the literature survey only revealed the following work.

Literature Survey

A. Yaneva et al\(^1\) separated strychnine from elixirs by TLC using ethyl alcohol, chloroform as mobile phase. C. Muller et al\(^2\) developed a gas chromatographic method for estimation of strychnine. M. Chiarotti et al\(^3\) developed capillary gas chromatography to estimate strychnine. A. Lawrence et al\(^4\) developed HPLC to estimate strychnine. Iskander et al\(^5\) developed HPLC for brucine.

Experimental

The label claimed per pill as,
Nux Vomica powder — — — 65mg.

Preparation of Test Solution

20 pills were weighed and powdered. Powder equivalent to the weight of 10 pills was weighed and transferred to a 250ml beaker. To this 80ml of ammoniacal chloroform was added and stirred with the help of a magnetic stirrer for about 10 minutes. The chloroform layer was removed. The residue was washed with 5ml each of chloroform 3-4 times and added to original layer. The chloroform layer was evaporated to dryness. The residue so obtained was dissolved in 50ml of chloroform and transferred to a 100ml volumetric flask. The volume was then made upto 100ml with chloroform.

Preparation of standard solution

7mg of drug strychnine and 8mg of brucine were weighed accurately and dissolved in about 50ml of chloroform. This solution was then transferred into a 100ml volumetric flask and the volume was adjusted with chloroform.

Separation and Quantitation of Alkaloids:

The chromatplates of 20X20 cm size were prepared with silica gel G\(^6\) of thickness 500 and then activated at 105-110\(^\circ\)c for 1 hr. The dried plate was divided into three parts, the central one kept for blank while the remaining for test and standard solutions each. Likewise three chromatplates were streaked using 0.25, 0.50, and 0.75ml of test and standard solution. The plates were developed with the mobile phase, Ethyl-acetate: Chloroform: Ammonia solution (40:8:2) in an unsaturated chamber and run to a distance of 13 cm. Visualisation was done by spraying the plate with acidified iodoplatinate reagent. For the purpose of scrappping reference plate with the same conditions was prepared and Visualised by above method and then knowing the Rf value,
scraping was done. The Rf values for strychnine and brucine were 0.72 and 0.92 respectively. The corresponding bands were scrapped out and then analysed by Shimadzu–UV 240/visible spectrophotometer at 251nm for strychnine and 267nm for brucine in ethyl alcohol.

**Recovery Experiment**

To the powder equivalent to weight of 10 pills, 10mg each of strychnine and brucine were added. From this admixture a quantity of powder equivalent to weight of 10 pills was analysed by the proposed method. The percentage recovery for both the alkaloids was obtained. Further statistical evaluation indicated the precision of the proposed method.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Content per pill by the proposed method (mg)</th>
<th>Amount of drug added (mg)</th>
<th>Amount of drug recovered (mg)</th>
<th>Percentages recovery</th>
<th>Standard deviation</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strychnine</td>
<td>0.7550</td>
<td>10</td>
<td>9.820</td>
<td>98.20</td>
<td>± 0.1979</td>
<td>2.6627</td>
</tr>
<tr>
<td>Brucine</td>
<td>0.8794</td>
<td>10</td>
<td>10.011</td>
<td>100.11</td>
<td>± 0.2052</td>
<td>2.3836</td>
</tr>
</tbody>
</table>

**Results and conclusion**

The samples of Nux Vomica powder from two different companies were analysed by the proposed method for the content of strychnine and brucine. The amount found is as follows.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Strychnine %</th>
<th>Brucine %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.2405</td>
<td>1.3886</td>
</tr>
<tr>
<td>II</td>
<td>1.1422</td>
<td>1.2232</td>
</tr>
</tbody>
</table>

**Acknowledgement**

The authors are thankful to Prof. J. Emmanuel, Ag. Principal, Goa College of Pharmacy, Panaji for providing the necessary facilities for this research work.

**REFERENCES**

DETERMINATION OF BROMHEXINE HCL IN TS FORMULATIONS BY COLORIMETRY

R. V. Gaitonde & Ganadlilsh J. Kamat
Goa College of Pharmacy, Panaji, Goa-403 001.

Abstract:

Bromhexine hydrochloride a primary aromatic amine was coupled with thymol after diazotizing it with sodium nitrite and hydrochloric acid. Quantitation was done by absorptiometric measurement of the orange dye at 450 nm which is the wavelength of its maximum absorbance. Validity of Beer's Law over a concentration range of 1—9 mcg/ml. made it possible to analyse the formulations of the drug by the proposed method after its standardisation. The results were statistically verified.

Bromhexine hydrochloride (BXH), chemically known as 2-amino-3,5-dibromo-N-cyclohexyl-N-methyl-benzene methanamine monohydrochloride is a potent mucolytic expectorant, official in B.P. 80. Official method is non-aqueous titration for the pure drug and U.V. spectrophotometry for tablets. Other analytical methods include U.V. spectrophotometry, gravimetry, titrimetry, GLC, HPLC. Bowtie et.al. described a colorimetric method based on extraction of ion pair complex with bromocresol purple. Maria Ines et.al. and Shingabl et. al employed Bratton Marshall reagent in its estimation.

The proposed method is based on coupling of diazonium salt of the drug with thymol in alkaline medium. The orange chromophore formed is stable for more than six hours and shows peak absorbance at 450 nm. Compliance with Beer's law is obvious from the linearity of plot of absorbance v/s concentration over the range of 1-9 mcg/ml.

Experimental:

Instruments:— 1) SHIMADZU UV 240, U.V./Visible recording spectrophotometer, Japan.

2) CZ Spekol spectrocolorimeter.

Reagents:

1. Solution of sodium nitrite (G.R.)—A 2% W/V solution in distilled water.

2. Solution of hydrochloric acid (Analar)—A 2% V/V solution of hydrochloric acid (36%) in distilled water.

3. Solution of ammonium sulphamate (BDH)—A 5% W/V solution in distilled water.

4. Solution of thymol—0.1% W/v solution in methanol (Analar).

5. Solution of sodium hydroxide (G.R.)—1% W/V solution in distilled water.

A reference sample of BXH was analysed as per B.P. 80 to check its purity. Accurately weighed 20 mg of the drug were dissolved in distilled water to 100 ml of solution. 25 ml of this solution after mixing were the diluted to 100 ml with same solvent to obtain a working standard containing 50 mcg/ml of BXH.

Preparation of Standard Drug Solution:

1. Tablets: 20 tablets were accurately weighed and finely powdered. Powder equivalent to 20 mg of the drug was extracted with four 20 ml portions of distilled water, passing each extract through a filter paper into a 100 ml volumetric flask. 10 ml of water was passed through the filter and final volume was made up with distilled water. 25 ml of this solution was diluted to 100 ml with distilled water.

2. Liquid Formulations: Amount of formulation equivalent to 20 mg of BXH was transferred to a separating funnel containing 5 ml of 1N sodium hydroxide solution and 25 ml of water. The solution was extracted with four 25 ml portions of chloroform. The combined chloroform extract was evaporated to dryness, residue dissolved in 25 ml of 0.1N hydrochloric acid and diluted to 100 ml with distilled water and mixed. 25 ml of this solution was further diluted with distilled water to 100 ml to obtain the sample solution. Assay Procedure:

1. 2 and 3 ml aliquots of sample solutions were transferred to different 25 ml volumetric flasks followed in
succession by 1.5 ml of dilute hydrochloric acid and 1.25 ml of solution of sodium nitrite. The solutions were mixed and allowed to stand for 10 min. 1 ml of solution of ammonium sulphamate was then added to each of the flasks followed by shaking till the evolution of gas ceased. 0.1 ml of solution of thymol followed by 2.5 ml of solution of sodium hydroxide were then added to each flask and volume was made up with distilled water. The absorbance of the solutions was read at 450 nm against the reagent blank prepared concomitantly. Concentrations of the solutions were computed from the calibration curve obtained by treating known amounts (0.5–6.0 inl) of standard drug solution as above and plotting absorbance as function of concentration.

The assay procedure includes the optimum reaction conditions which were derived by studying the effects of various parameters on the reaction. The method was subjected to recovery studies and statistical validation, results of which confirmed the reproducibility and reliability of the method. All the results are summarised in Table 1.

The authors are thankful to Principal J. Emmanuel for providing necessary facilities and Prof. O.S. Kamalapurkar for his contributions to this project.

REFERENCES

Analysis of Drug Preparation Containing Chlorpropamide and Phenformin Hydrochloride by Thin-Layer Chromatography

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Phytochemistry Laboratory, Goa College of Pharmacy, Panjim - Goa, 403001 India

KLORPROPAMID VE FENFORMIN-HCI İÇEREN TABLETLERIN İNE TABAKA KROMATOGRAFİSİ YÖNTEMIYLE ANALİZI

Özet


Summary

The pharmaceutical preparations are usually analyzed by pharmacopeial methods for their individual components. This involves either sophisticated instruments or time consuming factors. Therefore a method was developed to analyze a pharmaceutical antiidiabetic preparation in the form of a tablet containing chlorpropamide and phenformin hydrochloride. This involves, first, separation of the individual components by a simple thin-layer chromatography (TLC), and then estimating the same by spectrophotometric method (Shimadzu UV 240). Literature survey does not reveal any work on such above mentioned formulation. Therefore, the devised method was found simple, economical and time saving.

Keywords: Chlorpropamide - Phenformin HCl - TLC analysis - Spectrophotometric method

Adli Tip Derg., 3: 30 - 33 (1987)
INTRODUCTION

Mill and Chamberlain (1) determined phenformine in human body fluids by using HPLC. Allesandro et al (2) have estimated biguanides by complexometric titration. Their determination was by formation of Cu complexes from cuprammonium or Fehling's solution. Wickramsingha and Shaws (3) estimated phenformine and other biguanides by using gas chromatography. Joshi (4) determined chlorpropamide and its metabolites in plasma by using HPLC. Takla and Joshi (5) identified, assayed and determined the purity of chlorpropamide, glibenclamide and tolbutamide by TLC. They have used cyclohexane : chloroform : acetic acid : ethanol (10/112/1). Zecca and Colombo (6) estimated glibenclamide, chlorpropamide and tolbutamide in plasma by HPLC with UV detection.

MATERIAL AND METHODS

The label claims per tablet as: chlorpropamide 50 mg; phenformin 25 mg.

Preparation of test solution

Five tablets were triturated in a glass mortar, and then the powder equivalent to two tablets was extracted in 70 mL ethanol. The solution was filtered through Whatman no. 42 paper in a 100 mL volumetric flask. The residue was washed with 5 mL each of ethanol 4 times and then added to the original to make up the volume.

Preparation of standard solution

100 mg of chlorpropamide and 50 mg of phenformin were weighed accurately and dissolved in 100 mL of ethanol. The above samples were checked for its purity by IP specifications (Figures 1,2).

Separation and quantitation

The chromoplates of 20x20 cm size were prepared with silica gel G of thickness 500 μm and then activated at 100-105°C for one hour. Each three activated chromoplates were taken and streaked using 1, 1:25 and 1.5 mL of test and standard solution. The plates were developed in a saturated developing chamber using methanol : water : ammonia solution (49:49:2) as a mobile phase. The plates were run to a 12 cm which took 25 minutes. Visualization was done by spraying the plates with 2% potassium permanganate reagent in water. For the purpose of scrapping a reference plate under the same conditions was prepared and then knowing the RF values scrapping was done.
Figure 1. Spectra of chlorpropamide and phenformin.

Table 1. TLC - data of the analysis of chlorpropamide and phenformin HCl containing tablets.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Content/tab. by proposed method</th>
<th>Drug added (mg)</th>
<th>Drug recovered (mg)</th>
<th>Recovery (%)</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpropamide</td>
<td>54.08</td>
<td>100</td>
<td>102.6</td>
<td>102</td>
<td>0.3836</td>
<td>0.7080</td>
</tr>
<tr>
<td>Phenformin-HCl</td>
<td>27.42</td>
<td>50</td>
<td>51.9</td>
<td>103</td>
<td>0.3919</td>
<td>1.4453</td>
</tr>
</tbody>
</table>

The drugs were separated at following RF values:

Chlorpropamide 0.85, Phenformin 0.25.

The resulting band each of above test and standard solution was scrapped out and treated in the following manner:

a) Scraping corresponding to ref. chlorpropamide spot was extracted in 100 ml 0.01N HCl and then analyzed by Shimadzu UV 240 / Visible spectrophotometer at 232 nm (i.e. 1% 1 cm 600).
b) Scraping corresponding to ref. phenformin spot was extracted in 5 mL of 0.1N sulfuric acid and then analyzed by Shimadzu UV 240/Visible spectrophotometer at 251 nm (E 1 % 1 cm 11).

RESULTS AND DISCUSSION

The content of two tablets 100 mg chlorpropamide and 50 mg phenformin were added. From this mixture the quantity of content equivalent to 100 mg of chlorpropamide was taken and analyzed by proposed method (Table 1). The percentage recovery of the active ingredients was computed from the results obtained. Further statistical evaluation indicates the precision of the proposed method.

Acknowledgement

The authors are very much thankful to Prof. J. Emmanuel, Principal, Goa College of Pharmacy for providing necessary facilities for the research work.

REFERENCES


Reprints request to :

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TLC - Spectrophotometric Determination of Total Sennoside from Marketed Galenic Preparation

R.V.GAIKONDE, ULHAS BHAT

Phytochemistry Laboratory, Goa College of Pharmacy, Panaji, Goa - 403 001, India.

Summary

Galenical preparation sold in the market do not mention the amount of active ingredient having therapeutic effect. Such one preparation containing extract of Sonamukhi (*Cassia angustifolia*) was analysed for total sennoside content. The present work deals with finding out the actual amount of the active ingredient present there in, by TLC-spectrophotometric method.

Literature survey does not reveal any work on such a product.

Keywords: Galenical preparations - Active ingredient - Sennoside - TLC-Spectrophotometric method

Literature Survey

*Lane* (1) determined sennoside A and its derivatives in biological tissues by fluorometry. The fluorescence intensity was measured at 510 nm.

*Method Brendel and Schneider* (2) determined sennosides in senno pods and leaves spectrophotometrically. *Wahbi et al* (3) determined sennosides from senna powder by a colorimetric method. The yellow colour was measured at 390 nm.

*Hayashi et al* (4) determined sennosides in senna powder by HPLC.
Experimental

The label claimed on the galenical preparation, each 30 mL contains: extract of Sonamukhi - 1.15 gm.

Preparation of Test Solution

20 mL of galenical solution was diluted with hot distilled water and volume was made up to 100 mL.

Standard Solution

100 mg of the powder of calcium sennoside (20%) was digested with 70 mL of hot water, filtered and the volume was made up to 100 mL.

Separation and Quantitation of Sennoside

Chromoplates of 20 x 20 cm size were prepared with silica gel G of thickness 500 µm and then activated at 105°C - 110°C for one hour. Each three activated chromoplates were taken and streaked using 0.25, 0.50 and 0.75 mL of test and standard solution. The plates were developed in a saturated developing chamber using benzene: acetic acid (70:30) as a mobile phase. The plates were run to a 10 cm which took 30 minutes. Visualization was done by spraying with strong ammonia solution. For the purpose of scrapping a reference plate under the same conditions was prepared and then knowing the Rf value scrapping was done. The Rf value for sennoside was 0.9. The corresponding band was scraped out and analysed by Shimadzu UV 240/visible spectrophotometer at 270 nm in 5% bicarbonate solution.

Recovery Experiment

To 20 mL of the galenical solution 50 mg of powder of calcium sennoside (equivalent to 10 mg) was added. From this admixture the quantity of galenical 13.18 mL (equivalent to 19.32 mg of sennoside) was removed as per the results of preanalysed sample. The solution was then diluted with hot water and analysed by the proposed method. The percentage recovery was computed from the results obtained. Further, statistical evaluation indicated the precision of the proposed method.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sennoside</th>
<th>Amt. of drug added in mgs.</th>
<th>Amt. of drug recovered in percentage</th>
<th>Standard deviation (S)</th>
<th>Co-efficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sennoside</td>
<td>29.119 mg</td>
<td>10</td>
<td>98%</td>
<td>± 0.0099</td>
<td>1.02258</td>
</tr>
</tbody>
</table>
Acknowledgement

The authors were thankful to Prof. J. Emmeanuel, Principal GOA College of Pharmacy, Panaji, for providing the necessary facilities for this research work.

REFERENCES


Reprints request to:

R.V. Gaitonde
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ANALYSIS OF A DRUG PREPARATION CONTAINING EPHEDRINE HYDROCHLORIDE, THEOPHYLLINE, CHLORPHENIRAMINE MALEATE AND DIAZEPAM BY THIN LAYER CHROMATOGRAPHY.

R. V. Gaitonde* and Umesh Rivankar

(Received 22 October 86)

ABSTRACT

Marketed samples are usually analysed by official methods of Pharmacopoeia which are time consuming and tedious. Hence analytical study was carried out to evaluate a marketed sample containing Ephedrine, theophylline, chlorpheniramine maleate, Diazepam as their individual contents using thin layer chromatography and spectroscopy as analytical tool.

METHODS

Experimental:

Label claims, per tablet as

- Ephedrine Hydrochloride ... 20 mg
- Theophylline ... 100 mg
- Chlorpheniramine maleate ... 3 mg
- Diazepam ... 2.5 mg

Preparation of Test Solution:

Five tablets were triturated in a glass mortar and then powder content equivalent to two tablets were extracted in 70 ml ethanol. The solution was filtered through whatman no. 42 paper in a 100 ml volumetric flask. The residue was washed with 5 ml each of ethanol 4 times and the washings added to the original extract and the volume, made up to 100 ml with ethanol.

Preparation of Standard Solution:

40 mg Ephedrine Hydrochloride, 200 mg Theophylline, 6 mg Chlorpheniramine Maleate and 5 mg Diazepam were weighed accurately and dissolved in 100 ml ethanol.

The above samples were checked for their purity by I.P. specifications.

It was found that the absorbance vs concentration was linear for Ephedrine from 40 mcg to 80 mcg at 251 nm as in Graph (1) and for Theophylline from 5 mcg to 25 mcg at 270 nm as in Graph (2). It was confirmed for Chlorpheniramine Maleate from 5 mcg to 25 mcg at 265 nm as in Graph (3) and for Diazepam from 1 to 5 mcg at 241 nm as in Graph (4).

Separation and Quantitation:

The chromplates of 20 x 20 cm size were prepared with silica gel G of thickness 500 μ and then activated at 105 — 110°C for 1 hour. Each three chromplates were streaked using 1, 1.25 and 1.5 ml of Test and Standard Solutions. The plates were developed then by using the solvent system: Methanol : Chloroform : Strong Ammonia solution (50 : 15 : 1.5).
Visualisation was done by spraying the plate with 2% potassium permanganate reagent in water. For the purpose of scraping, reference plate under the same conditions was prepared and then knowing the Rf value scraping was done. The drugs were separated at the following Rf values:

- Ephedrine Hydrochloride \[ R_f = 0.5 \]
- Theophylline \[ R_f = 0.87 \]
- Chlorpheniramine Maleate \[ R_f = 0.75 \]
- Diazepam \[ R_f = 0.95 \]

The resulting band of each of the above test and standard solution was scraped out and treated in the following manner.

a) Scraping corresponding to Ref. ephedrine hydrochloride spot, was extracted in 5 ml 0.1 N \( \text{H}_2\text{SO}_4 \) and absorption measured on Shimadzu UV240/Visible Spectrophotometer at 251 nm (E 1% 1 cm. = 9).

b) Scraping corresponding to Ref. Theophylline Spot, was extracted in 20 ml 0.1 N HCl. From this 1 ml was taken and further diluted to 10 ml with 0.1 N HCl. The absorption of this was then measured on Shimadzu UV vis/visible Spectrophotometer at 270 nm (E 1% 1 cm = 530).

c) Scraping corresponding to reference Chlorpheniramine Maleate Spot, was extracted in 5 ml of 0.1
<table>
<thead>
<tr>
<th>Drug</th>
<th>Content/ tab by proposed methods in mgs.</th>
<th>Amount of drug added in mgs.</th>
<th>Amount of drug recovered</th>
<th>Percentage recovery</th>
<th>Std. deviation</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephedrine Hydrochloride</td>
<td>17.2</td>
<td>40</td>
<td>38.41</td>
<td>96%</td>
<td>0.2880</td>
<td>1.6808</td>
</tr>
<tr>
<td>Theophylline</td>
<td>95.9</td>
<td>200</td>
<td>194.45</td>
<td>97.22%</td>
<td>0.8000</td>
<td>0.8350</td>
</tr>
<tr>
<td>CPM</td>
<td>2.65</td>
<td>6</td>
<td>5.56</td>
<td>5.56%</td>
<td>0.7190</td>
<td>2.7051</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2.2</td>
<td>5</td>
<td>4.62</td>
<td>92.4%</td>
<td>0.0676</td>
<td>3.0459</td>
</tr>
</tbody>
</table>

NH₄SO₄. The absorption of this solution was measured on Shimadzu UV/visible Spectrophotometer at 265 nm. (E 1% 1 cm = 240).

d) Scraping corresponding to reference Diazepam Spot, was extracted in 20 ml of 0.1 N Sulphuric acid. The absorption was then measured on Shimadzu UV/visible Spectrophotometer at 241 nm (E 1% 1 cm = 1402).

Recovery:

To the content of 2 tablets, 40 mg of Ephedrine Hydrochloride, 200 mg of Theophylline, 6 mg of Chloropheniramine Maleate and 5 mg of Diazepam were added. From this mixture the quantity of content equivalent to 200 mg theophylline was taken and analysed by proposed method. The percentage recovery of the active ingredients were computed from the results obtained. Further statistical analysis indicated the precision of the proposed method.

ACKNOWLEDGEMENT

The authors are very much thankful to Prof. J. Emmanuel, Principal, Goa College of Pharmacy for providing necessary facilities for this research work.

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