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ANALYSIS OF SOME ANTI-INFLAMMATORY ARYLACETIC ACID DERIVATIVES THROUGH THE GRIESS REACTION

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ABSTRACT

A simple, rapid and accurate method for the spectrophotomatric determination of six anti-inflammatory arylacetic acid derivatives (Ibuprofen, naproxen, ketoprofen, indomethacin, pirprofen and flurbiprofen) in either the drug substances or in pharmaceutical formulations is proposed. The method is based on the development of the characteristic orange Griess pigment with sodium nitrito p-nitroaniline and 1-naphthylamine. A linear correlation was found between absorbance at λ_{max} of 470 nm and concentration . The colour is well developed by heating the mixture of the drug and reagents on a water-bath at 60°C for 15 minutes. The resulting chromogen is stable for at least 12 hours. Results of analyses of the official (first three) compounds is bulk drugs and dosage forms are in good agreemert with those of the B.P. 1980 procedures.

INTRODUCTION

Arylacetic acid derivatives are non-steroidal agents possessing a high degree of analgesic, anti-inflammatory and antipyretic activity and receiving the most intensive attention for clinical application. They are now widely used in the treatment of rheumatoid arthritis, osteoarthrosis, dental and postoperative pains and certain fevers.

Methods currently used for the quantitative analysis of arylacetic acid derivatives include titrimetry[1,2], polarography[3,4], UV-spectrophotometry[5,6], visible spectrophotometry[7], fluorimetry[9], gas-liquid chromatography[2,10,12] and high-performance liquid chormatography[2,13,14].

In the present work, six of the widely used anti-inflammatory arylacetic acid derivatives are subjected to an analytical study using the Griess reaction. These are ibuprofen. naproxen, indomethacin, ketoprofen, pirprofen and flurbiprofen. The nitrosated species used is p-nitroaniline (PNA) and the coupling reagent is 1-naphthylamine (1-NA). These are chosen on the basis of a published investigation on the kinetics and mechanism of the Griess reaction giving a comparison of different amine-coupler combinations and absorptivities of the resulting chromophores[15]. The proposed analytical method offers advantages of simplicity , rapidity and accuracy over many of the current methods.

EXPERIMENTAL

Materials :

Pure samples of ibuprofen, naproxen, indomethacin, ketoprofen, pirprofen and flurbiprofen were used as working standards. The commercial pharmaceutical dosage forms analysed are :

1- Brufen tablets (Kahira-Egypt U.L. of Boots-England) containing 200 mg of ibuprofen per tablet.

2- Brufen tablets containing 400 mg of ibuprofen per tablet.

3- Naprosyn tablets (Misr-Egypt U.L. of Syntex-England) containing 250 mg of naproxen per tablet.

4- Indocid capsules (Kahira-Egypt U.L. of Merck Sharp & Dohme-U.S.A.) containing 25 mg of indomethacin per capsule.

5- Profenid capsules (Alexandria Pharm. Co-Egypt U.L. of Rhone Poulenc-France) containing 50 mg of ketoprofen.

6- Profenid injection (Rhone Poulenc, Paris, France) containing 100 mg of ketoprofen per vial.

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7- Rengasil capsules (Swisspharma-Egypt U.L. of Ciba Geigy- Switzerland) containing 200 mg of pirprofen per capsule.

8- Rengasil ampoules cntaining 400 mg of pirprofen per ampoule.

9- Froben tablets (Boots, England) containing 100 mg of flurbiprofen per tablet.

Reagents :

1- Sodium nitrite solution, 2 % w/v in distilled water.

2- PNA solution, 2% w/v in methanol.

3- I-NA solution, 2% w/v in methanol.

All solvents used were of spectral grade.

Apparatus :

1- Unidec-320 spectrophotometer, JASCO, Tokyo, Japan.

2- Thermostatically controlled mLw water-bath, GDR.

Preparation of Working Standards :

Dissolve 50 mg of the appropriate working standard in 25 ml of methanol (in case of pirprofen dissolve the powder in 10 ml of petroleum ether and then complete to the mark with methanol). Dilute this solution to contain 0.2 mg ml^{-1} (for ibuprofen and pirprofen) or 0.4 mg ml^{-1} (for ketoprofen, indomethacin, naproxen and flurbiprofen). Use 1.0 ml of the final solution for colour formation with PNA and 1-NA solutions as directed under procedure.

Preparation of Samples :

Tablets : Weigh and powder 20 tablets. Extract a quantity of the powder equivalent to 50 mg of the anti-inflammatory drug twice with 20 ml of methanol by shaking for 30 minutes. Add sufficient methanol to produce 50 ml and filter. Complete as under preparation of working standards begining with :"Dilute this solution.....".

Capsules : Extract a quantity of the mixed contents of 20 capsules equivalent to 50 mg of the anti-inflammatory drug twice with 20 ml methanol by shaking for 30 minutes, add sufficient methanol to produce 50 ml and filter if necessary. In case of rengasil capsules exract the powder twice with 10 ml of petroleum ether and complete with methanol to 50 ml. Complete as under preparation of working standards begining with: "Dilute this solution .....".

Injections : For ampoules dilute the injection solution with methanol to contain 0.2 mg ml^{-1} of the arylacetic acid derivatives and then follow the coming procedure. For vials, dissolve the contents of the vial in 25 ml of methanol and dilute the resulting solution with methanol to contain 0.4 mg ml^{-1} of the drug. Use 1.0 ml of the prepared solution for colour formulation with PNA and 1-NA as directed under procedure.

Procedure :

Pipette 1.0 ml of the assay solution into a 10-ml volumetric flask, add 1.0 ml of sodium nitrite solution, 0.1 ml of PNA solution and 0.1 ml of 1-NA solution and mix thoroughly. Heat the mixture on a water-bath at 60°C for 15 min., cool to room temperature, and complete with aceto-nitrile to the mark. Measure the absorbance of the solution at 470 nm against a blank similarly treated substituting sample solution with 1.0 ml of methanol. The concentration of the assay solution is found from a properly constructed calibration graph.

RESULTS AND DISCUSSION

Reaction Involved :

It is now thought that the principal mechanism of action of this group of anti-inflammatory analgesics is the strong inhibition of the conversion of arachidonic acid into prostaglandin E_2 through the acidic function[16]. Therefore in this paper, the carboxylic group has been chosen as an analytical "handle" for the quantitative estimation of these

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compounds. A sensitive reaction for this function is the "Griess reaction", the mechanism of which is summarized in scheme 1 [15,18,19]. The final Griess pigment has an absorption maximum at 470 nm.

Optimisation of Variables :

The conditions for the production of the most intense and stable colour were studied. Ibuprofen was studied as a representative example of arylacetic acid derivatives in the following investigations.

a- Effect of Nitrite Concentration :

The optimum concentration of aqueous sodium nitrite solution leading to maximum intensity of colour was found to be 1.0 ml of 2% sodium nitrite per 10 ml of the reaction mixture (Fig. 1). Higher nitrite concentrations did not increase the absorption intensity. The importance of control of nitrite concentration is readily apparent. In addition to the bimolecular reaction to form the nitrosating compound N_2O_3, nitrous acid undergoes a termolecular reaction to form NO and NO_3^-. Some nitrite may be also lost by reaction with 1-naphthylamine. The nitrosation of 1-naphthylamine is the basis for a method of nitrite determination[20].

b- Effect of PNA and 1-NA Concentrations :

It was found that the use of 0.1 ml of 2% methanolic solution of each of these reagents was sufficient for maximum colour development.

c- Effect of Heating Time :

Maximum absorption intensity was obtained after heating the reaction mixture on a water-bath at 60± 5°C for

15 minutes (Fig. 2). The coloured solution after dilution with solvent was stable for at least 12 hours.

d- Effect of Solvent :

The diluting solvents investigated were water,methanol, ethanol, propan-1-ol, propan-2-ol, acetone, acetonitrile, dimethyl sulphoxide and dimethylformamide. Acetonitrile gave the highest absorption intensity of the chormophore

e- Quantification, Accuracy and Precision :

Linear correlations were found between the absorbances of the coloured solutions at 470 nm and the concentration of each arylacetic acid derivative in the ranges given in Table 1. The apparent molar absorptivities as well as variables and descriptors of the resulting linear regression equations are given also in Table 1. The concentration of an unknown solution could be determined from calibration graphs properly constructed using authentic samples.

The reproducibility of the procedure was determined by running replicate samples, each containing 20 ug ml^{-1} (for ibuprofen and pirprofen) or 40 ug ml^{-1} (for ketoprofen, indomethacin, naproxen and flurbiprofen) in the final test solution. At these concentration levels, the relative standard deviations were 0.60%, 0.85%, 0.96 %, 0.55 %, 0.84% and 0.67% for ibuprofen, naproxen, indomethacin, ketoprofen, pirprofen and flurbiprofen respectively.

Application to Drug Substances and Dosage Forms :

The suggested method was applied to the quantitative determination of the investigated anti-inflammatory drugs in bulk and in pharmaceutical formulations (Table 2) .

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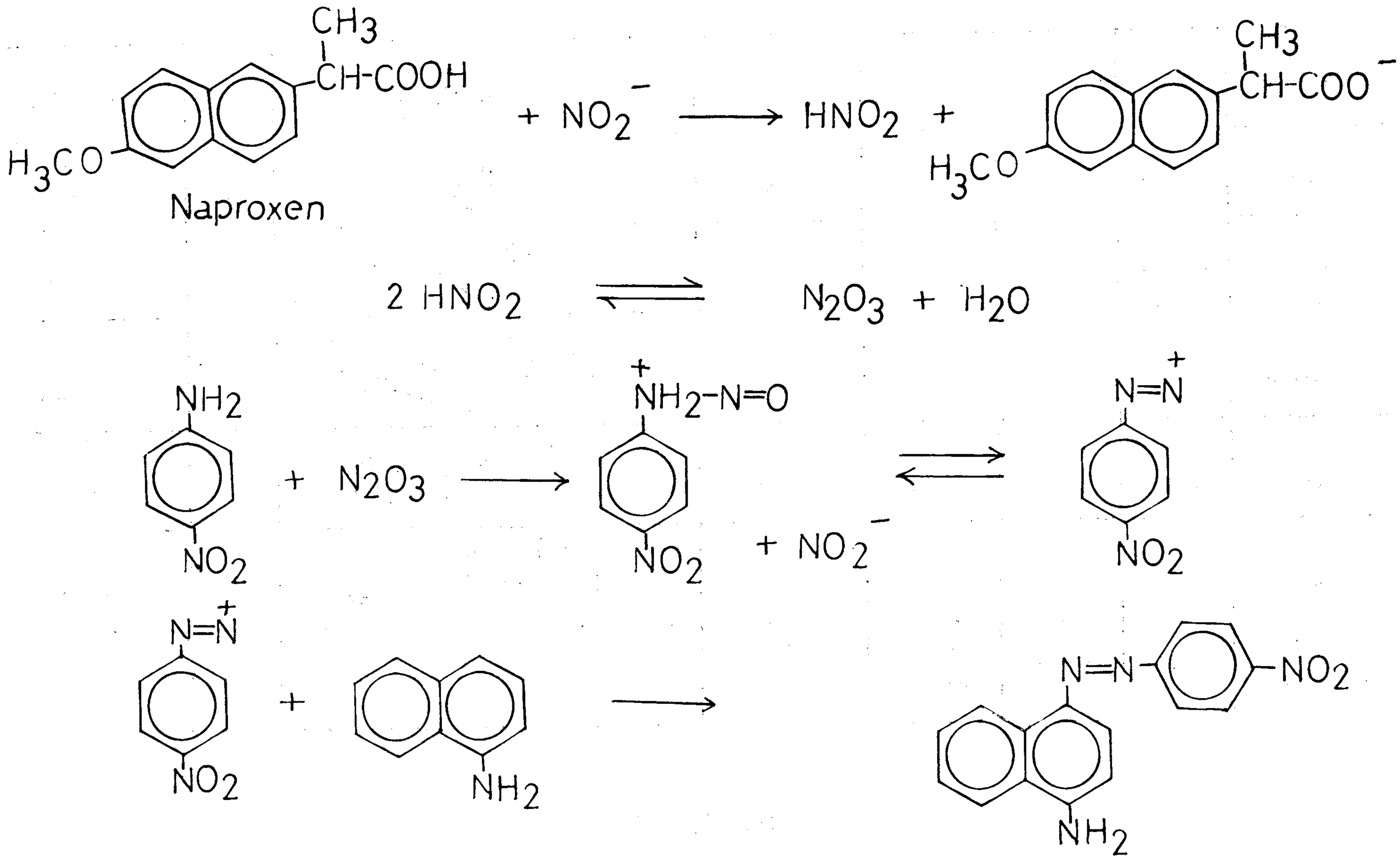
Results of analysis of the official drugs (ibuprofen , naproxen and indomethacin) in bulk and dosage forms agree well with those of the B.P. 1980 methods. For non-official drugs (ketoprofen, pirprofen and flurbiprofen) results of analysis are compared with the alkalimetric method recommended for ibuprofen by B.P. 1980.

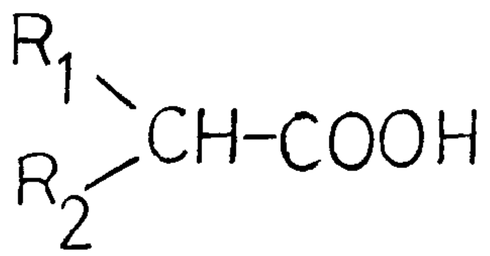
Student's t-and F-tests showed no significant difference between the proposed and compendial procedures. This indicate the suitability of the method for routine quality control analysis of the cited drugs.

It might be noted that any acidic compound existing in some pharmaceutical preparations like certain preservatives will exhibit serious interferences on carrying out this reaction.



Scheme 1. Reaction mechanism.

Table 1: Quantitative parameters for the assay of some arylacetic derivatives by the proposed method.



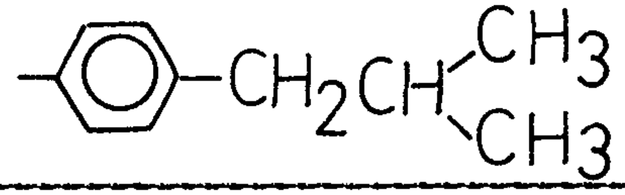
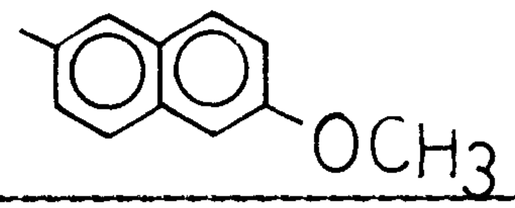
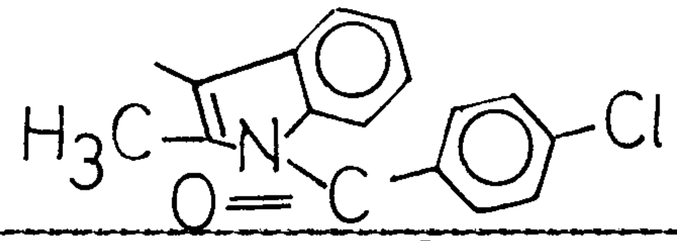
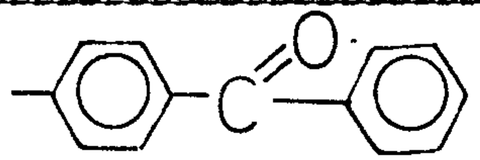
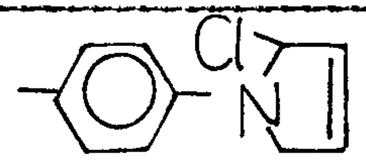
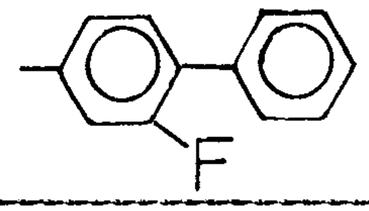
Compound	R_1	R_2	ε_{max}, 10^3 l mol^{-1}cm^{-1}	Linear range µg ml^{-1}	Intercept	Slope	Correlation coefficient
Ibuprofen	CH_3		7.57	5-30	-0.0774	0.0367	0.9999
Naproxen	CH_3		4.70	10-60	-0.1177	0.0204	0.9997
Indomethacin	H		3.79	10-100	0.0216	0.0106	0.9992
Ketoprofen	CH_3		4.65	10-60	0.0165	0.0183	0.9997
Pirprofen	CH_3		6.46	5-40	0.0179	0.0257	0.9991
Flurbiprofen	CH_3		3.93	10-60	-0.0005	0.0161	0.9999

Table 2 : Analysis of some arylacetic acid derivatives by the proposed and B.P. 1980 methods.

Sample	Proposed method		B.P.1980 method or alkalimetric method		t **	F(a)
	Mean Recovery * %	S.D %	Mean Recovery * %	S.D %		
Ibuprofen	99.7	0.60	100.0+	1.28	0.5190	4.55
Naproxen	100.1	0.85	99.9+	1.55	0.7342	3.33
Indomethacin	99.8	0.96	99.5+	1.46	0.4201	2.31
Ketoprofen	99.6	0.55	99.5++	1.22	0.1828	4.92
Pirprofen	99.7	0.84	99.9++	1.44	0.2936	2.94
Flurbiprofen	99.9	0.67	99.6++	1.50	0.4468	5.01
Brufen 200 tablets	97.8	0.72	98.0+	1.58	0.2818	4.82
Frufen 400 tablets	98.5	0.64	98.2+	1.39	0.4797	4.72
Indocid capsules	99.8	0.92	100.0+	1.12	0.3377	1.48
Profenid capsules	99.2	0.52	99.0++	1.14	0.3906	4.81
Profenid injection	100.2	0.61	99.9++	1.18	0.5527	3.74
Rengasil capsules	99.6	0.78	100.0++	1.52	0.5729	3.80
Rengasil ampoules	99.1	0.75	98.8++	1.42	0.4571	3.58
Naprosyn tablets	96.8	0.80	97.1+	0.98	0.5803	1.50
Froben tablets	99.4	0.72	99.0++	1.48	0.5947	4.23

* Mean of 6 determinations

\+ B.P. 1980 method (for official drugs).

++ Titration with 0.1 M NaOH using phenolphthalein as indicator (for non-official drugs) by the same method recommended for ibuprofen BP 1980.

** Calculated t for 10 degrees of freedom at p 0.05=2.2281

(a) Calculated F for (5,5) degrees of freedom at p 0.05=5.05.

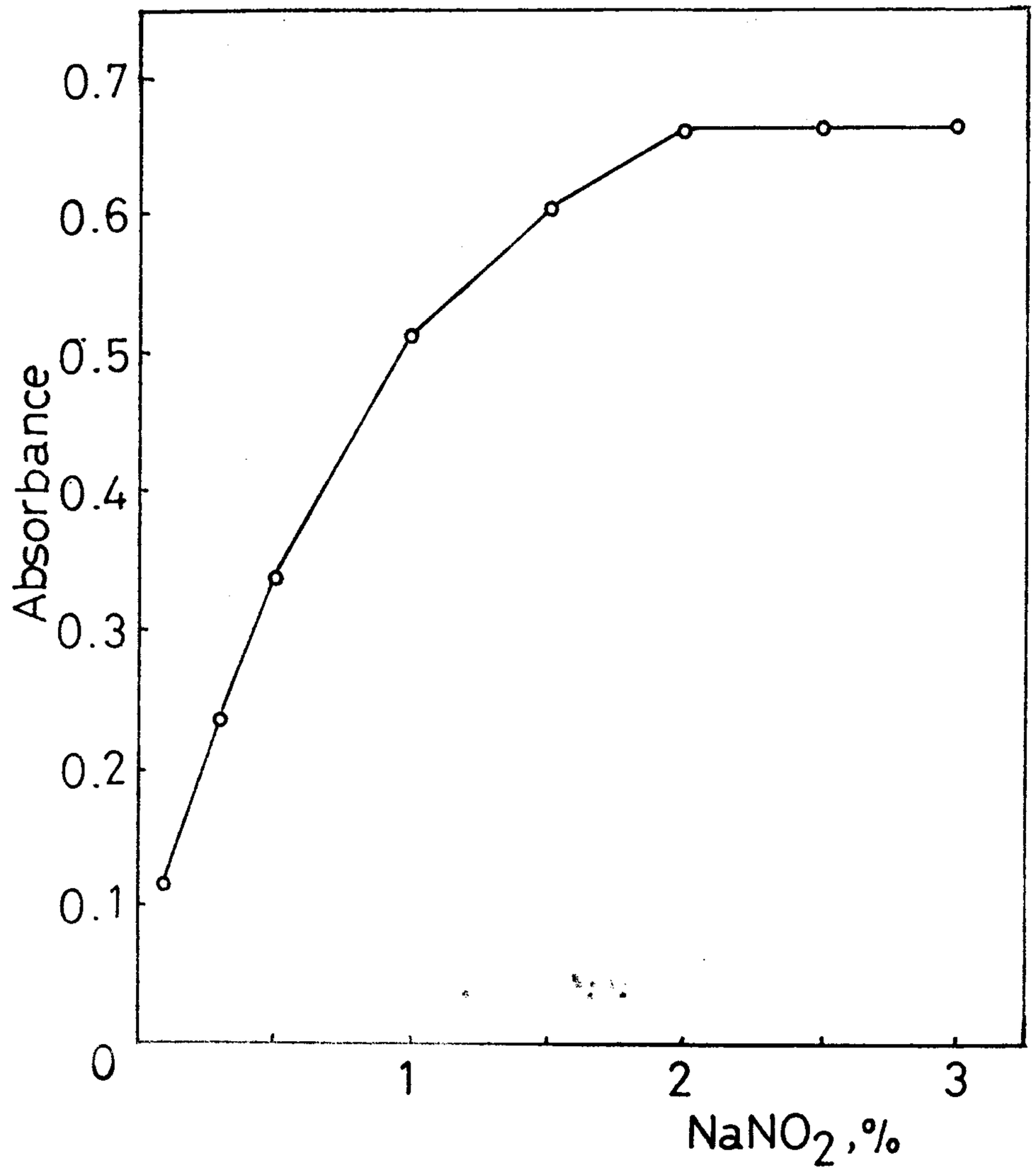


Fig. 1: Effect of sodium nitrite concentration on colour intensity. Concentration of ibuprofen in the final assay solution: 20 ug ml^{-1}.

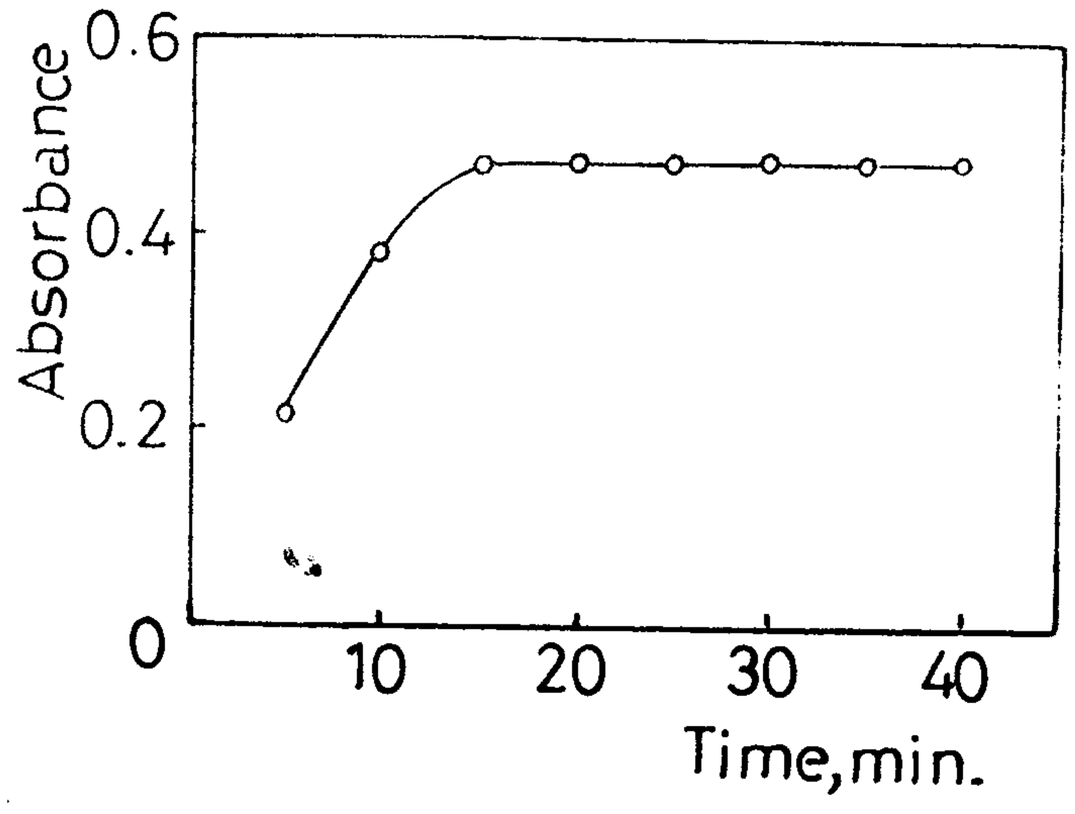


Fig. 2: Effect of heating time on colour intensity.
Final concentration: 20 μg ml^{-1} of ibuprofen.

REFERENCES

1- *British Pharmacopoeia 1980*, H.M. Stationery Office, London(1980), pp. 236,239,300,534,779,791.

2- United States Pharmacopeia XXI Revision, National Formularly XVI Edition, U.S. Pharmacopeial Convention, Rockville, U.S.A (1985), pp. 526-527,532-533, 710.

3- P. Populaire; B. Terlain, S.Pascal, B. Decouvelaire, G. Lebreton, A. Renard and J.P. Thomas; Ann. Pharm. Fr., 31 (11) 679 (1973).

4- G. Kazemifard and L.Holleck; Arch Pharm., 306 (9) 664 (1973).

5- A. Allesandro, F.Mari and S.Settcase; Boll. Chim. Farm., 105, 761 (1966).

6- B. Lotti; Boll. Chim. Farm., 114 (6) 351 (1975).

7- B. Unterhalt; Pharm. Ztg., 123, 1801 (1978).

8- K. P.R. Chowdary; G.N.Rao and V.B. Rao, Indian Drugs, 24 (7) 361 (1987).

9- E. Hridberg, H.H. Lausen and J.A. Jansen; Eur. J.Clin. Pharmacol., 4, 119 (1974).

10- P. Heusse and L. Raynaud; Ann. Pharm. Fr., 36 (11-12) 631 (1978).

11- M.A. Evans; J. Pharm. Sci., 69, 219 (1980).

12- P.H. Degen, A. Schweizer and A.Sioufi; J. Chromatorgr.,290, 33 (1984).

13- C.M. Moore and I.R. Tebbett; Forensic Sci., Int., 34 (1-2) 155 (1987).

14- V.A. Johnson and J.T. Wilson; J. Chromatogr., Biomed. Appl., 55, 367 (1986).

15- J.B. Fox; Anal. Chem.,51 (9) 1493 (1979).

16- T.Y. Shen; Angew Chem. (Internat. Ed.), 11, 460 (1972).

17- R. Nickander, F.G. Mc Mahon and A.S. Ridolfo;Ann. Rev.Pharmacol. Toxicol., 19, 469 (1979).

18- E.D. Hughes, C.K. Ingold and J.H.Ridd; J.Chem.Soc.,65(1958).

19- Y. Nomura, Bull.Chem. Soc. Japan, 32, 536 (1959).

20- L.Kamm, D.F.Bray and D.E.Coffin; J. Assoc.Off. Anal. Chem.,51,140(1968).

تحليل بعض مضادات الالتهاب من مشتقات حمض الخليك العطرية باستعمال تفاعل جرايس

ميشيل ايليا القمص ، كاملة محمود عمارة
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فى هذا البحث تم التوصل الى طريقة سهلة للتقييم الطيفى لستة مركبات مضادة للالتهاب من مشتقات حمض الخليك العطرية (ابيبروفين، نابروكسين ،اندوميثاسين ،كيتوبروفين ، بيربروفين ، فلوربيبروفين) وذلك فى المواد النقية والمستحضرات الصيدلية ، وتعتمد الطريقة على تكوين صبغة حرايس البرتقالية اللون التى تنتج عن تفاعل العقار مع نيتريت الصوديوم ونيتروالانيلين المقابل و ١ ـ نافتايلاميين .

وتتلخص الطريقة فى تسخين محلول العقار مع الكواشف على حمام مائى عند ٦٠°م لمدة ١٥ دقيقة وتظل الصبغة الناتحة ثابتة لمدة لاتقل عن ١٢ ساعة . وقد تمت دراسة كل العوامل التى تؤثر على شدة امتصاص الصبغة مثل تركيز الكواشف ومدة التسخين والمذيب المستخدم . ووجدت علاقات خطية بين تركيز العقار وشدة امتصاص اللون الناتج عند موجة طولها ٤٧٠ ن م وتم استنباط المعادلات الخطية التى تعبر عن هذة العلاقات .

وقد وجدت نتائج التحليل بهذة الطريقة متطابقة تماما مع نتائج طريقة دستور الادوية البريطانى لعام ١٩٨٠ بالنسبة للعقاقير المعتمدة بهذا الدستور.

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