CHAPTER 2

SPECTROPHOTOMETRIC DETERMINATION OF ANALGIN AND ITS DOSAGE FORMS

2.1 Introduction

Analgin, sodium N-(1,5-dimethyl-3-oxo-2-phenylpyrazolin-4-yl)-N-methylamine methane sulphonate monohydrate (dipyrone, metamizol, novalgin), is an analgesic and antipyretic drug. The risk of agranulocytosis in patients taking analgin is recognised. Its use is justified only in serious or life threatening conditions where no alternative antipyretic is available or suitable. It has been used in the treatment of muscular rheumatism, sciatica, lumbago, fibrositis neuritis, neuralgia and other painful conditions.

Analgin readily undergoes hydrolysis and oxidation according to the Scheme VI summarised by Pellerin and Letavernier. Yankova et al. isolated fifteen decomposition products from chloroform extract of yellow brown aqueous analgin solution obtained after storage for one year at room temperature. The degradation products have been identified by TLC, UV, IR, NMR and mass spectral
analysis. The degradation pathways are given in Scheme VI. In the solid state, analgin\textsuperscript{26} (15) is oxidized to 4-formylaminoantipyrine (16). In the acidic solution,\textsuperscript{26} analgin (15) is converted into 4-hydroxymethylmethylaminoantipyrine (17). In aqueous solution at neutral pH, analgin (15) is in equilibrium with sodium hydroxymethane sulphonate (19) and 4-methyleminoantipyrine (18).\textsuperscript{27} The compound (18) may be oxidized and hydrolysed by two possible routes. One pathway\textsuperscript{26-28} leads to the formation of methylrubazonic acid (24) through 4-aminoantipyrine (22) and iminobisantipyrine (23).

An alternative pathway\textsuperscript{28} leads to the formation of bismethylantipyrinylmethane (20) obtained from reaction of (17) and (18). Another possible degradation compound formed on prolonged storage has been identified\textsuperscript{25,26} as 4-hydroxyantipyrine (21). Compounds 18, 21 and 22 have also been identified as metabolites\textsuperscript{29} of analgin. Compounds 18 and 22 have been detected in parenteral solutions of analgin as the two main decomposition products.

The hydrolysis of analgin has been studied also in the pH range 1-12.\textsuperscript{30,31}
Scheme VI
SCHEME VI

18

\[ \text{H}_3\text{C} - \text{N} - \text{C}_6\text{H}_5 \] \[ \text{CH}_3 \]

\[ \text{H}_2 \] \[ \text{J}_2 \] \[ \text{Temperature} \]

\[ \text{H}_3\text{C} - \text{N} - \text{C}_6\text{H}_5 \] \[ \text{H}_2\text{C} - \text{N} - \text{C}_6\text{H}_5 \]

\[ \text{O}_2 \] \[ \text{hv} \]

22

\[ \text{H}_3\text{C} - \text{N} - \text{C}_6\text{H}_5 \] \[ \text{CH}_3 \]

\[ \text{H}_3\text{C} - \text{N} - \text{C}_6\text{H}_5 \] \[ \text{CH}_3 \]

23

24

\[ \text{H}_3\text{C} - \text{N} - \text{C}_6\text{H}_5 \] \[ \text{CH}_3 \]

\[ \text{H}_3\text{C} - \text{N} - \text{C}_6\text{H}_5 \] \[ \text{CH}_3 \]
2.1.1 Analysis of Analgin

Some microcolor tests\textsuperscript{32} have been described for analgin with ammonium molybdate, ammonium vanadate and ferric chloride. The assay procedures for analgin are based primarily on the reducing property of the bisulphite group or upon the color formation with hydrolysed product of analgin. The various methods for the analysis of analgin include titrimetric, spectrophotometric and chromatographic techniques.

2.1.1.1 Titrimetric Methods

Analgin after hydrolysis is titrated against standard iodine solution. The method is reviewed by Savelva.\textsuperscript{33} Iodine reacts only with bisulphite formed in the hydrolysis of analgin in acidic as well as in mild alkaline media. The procedure is official in the State Pharmacopoeia of USSR,\textsuperscript{34} Pharmacopoeia of India,\textsuperscript{35} and Hungarian Pharmacopoeia.\textsuperscript{36}

Analgin has been titrated in nonaqueous medium against acetous perchloric acid. The end point is detected visually or potentiometrically.\textsuperscript{37} Acetous hydrochloric acid is also employed as titrant.\textsuperscript{38}
Analgin has been determined by cerimetry. In the complexometric titration, it can be titrated with EDTA, p-phenylenediamine dihydrochloride and other reagents.

Volumetrically, analgin has been determined by titration with barium chloride solution after the oxidation of bisulphite to sulphate with hydrogen peroxide.

In the argentometric titration, excess of silver nitrate solution is added to analgin solution followed by back titration with ammonium thiocyanate.

2.1.1.2 Spectrophotometric Methods

Most of the spectrophotometric procedures are based on the formation of color chromogen when the reagent reacts with 4-aminoantipyrine or formaldehyde which is obtained on hydrolysis of analgin in aqueous solution.

Analgin in aqueous solution gives 4-aminoantipyrine which reacts with p-dimethylaminobenzaldehyde to produce a yellow colored Schiff base. The reaction forms the basis of spectrophotometric determination of analgin. Methods are reported in which p-dimethylaminocinnamaldehyde, 1,2-naphthoquinone-4-sulphonic acid and p-benzoquinone are used in the place of p-dimethylaminobenzaldehyde.
The procedures involving use of modified Grote's reagent, \textsuperscript{49} acid dye \textsuperscript{50} and reaction with phenol and potassium ferricyanide, \textsuperscript{51} citral and trichloroacetic acid in absolute methanol, \textsuperscript{52} citric acid in mixture with methanol and acetic anhydride \textsuperscript{53} have been proposed for the estimation of analgin.

In the presence of alkali, analgin reduces phosphomolybdic acid to molybdenum blue. This observation forms basis of the spectrophotometric determination of analgin. \textsuperscript{54} Analgin is treated with iodic acid and the liberated iodine is extracted with an organic solvent and measured spectrophotometrically. \textsuperscript{55,56}

A spectrophotometric method based on copper-analgin complex has been described. \textsuperscript{57,58} Analgin is determined colorimetrically by measuring the formaldehyde, liberated on hydrolysis, with chromotropic acid \textsuperscript{59} and Schiff reagent. \textsuperscript{60} The results obtained by chromotropic acid method are not reproducible. In Schiff reagent method, the reagent being unstable, fresh Beer's curve is required every time.

In other colorimetric methods, the color is developed by the reaction of analgin with 2,2'-bipyridine, \textsuperscript{61,62} neotetrazolium chloride, \textsuperscript{63} and uranyl nitrate reagent. \textsuperscript{64}
2.1.1.3 Chromatographic Methods

Pellerin and Letavernier have determined analgin by GLC technique. The main disadvantage of this method is the thermal decomposition of analgin occurring in the injector.

HPLC has been employed for the estimation of analgin and its degradation products in pharmaceutical formulations.

Quantitative thin layer chromatography (TLC) has been proposed for the determination of pure analgin and analgin in dosage forms. Column chromatography is also suggested for the separation and determination of analgin in combination drugs.

2.1.1.4 Miscellaneous Methods

The other methods for the determination of analgin include potentiometric, conductometric, coulometric, polarographic, ionexchange resins, UV spectrophotometric, gravimetric and phototurbidometric techniques.
2.1.2 Determination of Analgin using Hantzsch reaction

In the present work, a spectrophotometric method based on Hantzsch reaction is described.

Earlier, Nash\textsuperscript{18} has reported that formaldehyde in aqueous solution reacts with acetylacetone in the presence of ammonium acetate, to afford yellow colored, 3,5-diacetyl-1,4-dihydrolutidine. Analgin is known to liberate formaldehyde, sodium bisulphite and 4-methylaminoantipyrine on hydrolysis. Therefore, it was thought of interest to extend the application of the Hantzsch reaction to determine analgin in bulk powder and its dosage forms.

The reaction conditions are modified suitably to establish a method for the assay of analgin. The effect of various reaction conditions such as pH and time for hydrolysis of analgin, as well as pH, time, temperature of reaction, reagent and analgin concentration, etc. on the color intensity are standardized.

Pure samples of analgin are analysed by the proposed method and the results compare favourably with those obtained by the USSR Pharmacopoeia method.\textsuperscript{34}
The procedure is applied successfully to the analysis of analgin and its combination dosage forms.

The method is simple, rapid, precise and accurate.
2.2 **Experimental**

2.2.1 **Apparatus**

1. Double beam Beckman Model 25 spectrophotometer having two matched cells of 1 cm. light path.
2. Systronic pH meter.
4. Corning volumetric flask of 25 and 100 ml capacity.
5. TLC Plate (20 x 20 cm).

2.2.2 **Reagents and Materials**

Analgin IP recrystallised from ethanol; Paracetamol BP; Caffeine BP; Oxyphenbutazone BP; Dexamethasone BP; Phenylbutazone BP; Diazepam USP; Papaverin Hydrochloride USP; Atropine methonitate BP; Potassium Chloride AR (BDH); Disodium Hydrogen Phosphate (dihydrate) (BDH); Potassium Hydrogen Phthalate (BDH); Potassium Dihydrogen Phosphate AR (BDH); Sodium Hydroxide (Pellets) (BDH); Hydrochloric Acid (BP); Ammonium Acetate (BDH); Silica Gel G (BDH); Acetylacetone (SD'S); Formaldehyde (BDH); Ethanol (BP); and double distilled water were used in the study.

The dosage forms of analgin are procured from local market.
2.2.2.1 Preparation of Buffer Solution

The buffer solutions of pH 1.0 to 2.0 were prepared by mixing potassium chloride solution (0.2M) and hydrochloric acid (0.2M) solution.

The buffer solutions of pH 3.0 to 5.0 were prepared by mixing potassium hydrogen phthalate solution (0.2M) and hydrochloric acid solution (0.2M) or sodium hydroxide solution (0.2M).

The buffer solutions of pH 6.0 to 8.0 were prepared by mixing potassium dihydrogen phosphate solution (0.2M) and sodium hydroxide solution (0.2M).

The final pH of a buffer solution was adjusted on pH meter.

2.2.2.2 Preparation of reagent solution

The reagent solution was prepared by dissolving ammonium acetate (300g) and freshly distilled acetylacetone (4.0ml) in distilled water. The final volume was adjusted to one litre with water and stored in a refrigerator.
2.2.2.3 Preparation of standard analgin solution

Analgin (50mg) was weighed accurately and dissolved in and diluted to 50 ml with ethanol. An aliquot (5 ml) was diluted further to 50 ml with the same solvent. Final solution contained 100 mcg of analgin per ml of the solution.

2.2.2.4 Preparation of Simulated Gastric Juice
(modified from USP 62)

Simulated gastric juice was prepared by dissolving sodium chloride (2g), pepsin (3.2g) and hydrochloric acid (2ml) in water to make 1 litre. The pH of the solution was 1.8 ± 0.05 (37°C).

2.2.2.5 Preparation of standard formaldehyde solution

Accurately weighed formaldehyde solution (1.081g; 37% w/w) was dissolved and diluted to 100 ml with water. The solution (5 ml) was diluted further to 100 ml with the same solvent. An aliquot (5 ml) was further diluted to 100 ml with water. Final solution contained 10 mcg of formaldehyde per ml of the solution.
2.2.2.6 Preparation of standard 4-methylaminoantipyrine solution

4-Methylaminoantipyrine (50mg) was weighed accurately and dissolved in and diluted to 50ml with absolute ethanol. An aliquot (5ml) was diluted further to 50ml with same solvent. Final solution contained 100mcg of 4-methylaminoantipyrine per ml of the solution.

2.2.3 Procedure

2.2.3.1 Determination of wavelength of maximum absorbance

Standard analgin solution (5.0 ml) was pipetted into a 100-ml conical flask. The buffer solution (pH1; 5ml) was added to it. The reaction mixture was allowed to stand for 5 minutes at room temperature with occasional shaking. The reagent solution (5ml) was added to it and the reaction mixture was immersed in a boiling water bath for 10 minutes. It was cooled to room temperature. The contents of the flask were transferred quantitatively to 25-ml volumetric flask with the help of water. The volume was adjusted to the mark with water. The absorbance of the colored solution was scanned on Beckman Model 25 spectrophotometer in the range of 350 to 550 nm against
blank. The blank was prepared similarly in which volume of standard analgin solution was replaced by an equal volume of ethanol.

Maximum absorbance was obtained at 412 nm (Fig. 1).

2.2.3.2 Lambert-Beer's curve for Analgin

Standard analgin solution (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 ml) was pipetted into a series of 100-ml conical flasks and analyzed as described under 2.2.3.1.

The absorbance of the reaction mixture was measured at 412 nm against blank (Fig. 2).

2.2.3.3 Lambert-Beer's curve for Formaldehyde

Standard formaldehyde solution (0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml) was pipetted into a series of 100-ml conical flasks and analyzed as described under 2.2.3.1.

The absorbance of the reaction mixture was measured at 412 nm against blank (Fig. 3).
2.2.4 Factors affecting the development of color

2.2.4.1 Effect of pH on hydrolysis of analgin

Standard analgin solution (5.0 ml) was treated with buffer solutions of varying pH (pH 1.0 to 8.0; 5 ml) and allowed to stand for 5 minutes at room temperature with occasional shaking. The reagent solution (5 ml) was added to it and analyzed as described under 2.2.3.1. The absorbance of the colored solution was measured at 412 nm against blank. Maximum absorbance was observed in range 1.0 to 3.0 pH (Fig. 4).

2.2.4.2 Effect of buffer (pH 1) concentration on hydrolysis of analgin

Different volumes (1.0 to 10 ml) of buffer solution (pH 1) were pipetted into a series of 100-ml conical flasks containing standard analgin solution (5.0 ml) and analyzed as described under 2.2.3.1. The absorbance of the reaction mixture was measured at 412 nm against blank prepared as usual.

Maximum absorbance was observed in the presence of 5 ml buffer solution which remained constant on increasing the volume of buffer solution (Fig. 5). In the present work, 5 ml buffer solution was used.
2.2.4.3 Effect of time for hydrolysis of Analgin

Standard analgin solution (5.0 ml) was pipetted into a series of 100-ml conical flasks and buffer solution (pH 1; 5 ml) was added to each of them. The reaction mixtures were allowed to stand for 2, 3, 4, 5, 6, 7 and 8 minutes at room temperature with occasional shaking. Reagent solution (5 ml) was added to each flask and the reaction mixture was analyzed as described under 2.2.3.1. The absorbance of the colored solution was measured at 412 nm against blank prepared as usual.

Maximum absorbance was observed after 5 minutes which remained constant on further standing (Fig.6).

2.2.4.4 Effect of concentration of reagent solution

The absorbance of the colored product formed by the reaction of standard analgin solution (5.0 ml) after treatment with buffer solution (pH 1; 5 ml), for 5 minutes with different volumes of the reagent solution was measured at 412 nm.

Maximum color intensity was obtained in the presence of 4 ml of reagent solution which remained constant with further increase in the volume of reagent solution (Fig.7).
2.2.4.5 Effect of temperature on reaction

The reaction of standard analgin solution (5.0 ml) with reagent solution (5 ml) was carried out at 25°, 37°, 60° and 100°C as described under 2.2.3.1 for 10 minutes. Absorbance of the reaction mixtures was measured at 412 nm (Fig. 8).

Maximum color intensity was obtained at 100°C temperature.

2.2.4.6 Effect of time of heating

The standard analgin solution (5.0 ml) after the treatment with buffer (pH 1; 5 ml) for 5 min at room temperature was reacted with reagent solution (5 ml). The reaction mixture was immersed in a boiling water bath for different time intervals. The absorbance of reaction mixture was measured at 412 nm against blank.

Maximum color intensity was obtained after 6 minutes which remained constant on further heating (Fig. 9).

2.2.4.7 Stability of colored product

Analgin solution (5.0 ml) was reacted with the reagent (5 ml) as described under 2.2.3.1 to form a yellow
color product. One set of reaction mixture was stored in dark and another set was allowed to stand in diffuse sun light. After different time intervals of storage, the color intensity was measured at 412 nm (Fig. 10).

The absorbance of the reaction mixture stored in dark was constant for more than 24 hours, while that of the reaction mixture kept in diffuse sunlight was constant upto 5 hours.

2.2.4.8 TLC of analgin hydrolysate

Standard analgin solution (5.0 ml) was treated with buffer solution of varying pH 1 to 8 (5 ml) and was allowed to stand for 5 minutes at room temperature with occasional shaking. The hydrolysate (0.01 ml) and standard solution of 4-methylaminoantipyrine (0.01 ml) were spotted on plate, previously coated with silica gel-G and activated at 105°C for 1 hour. The chromatogram was developed in a solvent system consisting of ethanol : chloroform : benzene : 10% ammonia solution (5 : 3 : 1.8 : 0.2). The plate was dried in air and the spots were made visualized by exposing to iodine vapours (Fig. 11).
2.2.5.1 Analysis of analgin

Accurately weighed amount of analgin (ca. 50 mg) was dissolved in and diluted to 50 ml with ethanol. The solution (5 ml) was diluted further to 50 ml with the same solvent. The diluted solution (5.0 ml) was analyzed as described under 2.2.3.1.

The amount of analgin was determined by referring to the standard curve (Table I).

2.2.5.2 Analysis of analgin in combination with Paracetamol, Caffeine, Phenylbutazone, Oxyphenbutazone, Diazepam, Dexamethasone, Papaverine Hydrochloride and Atropine methonitrate in synthetic mixtures

The synthetic mixtures containing analgin and paracetamol, caffeine, phenylbutazone, oxyphenbutazone, diazepam, dexamethasone, papaverine hydrochloride or atropine methonitrate in proportion usually encountered in the formulation of dosage forms were prepared in laboratory (Table II). The mixture equivalent to analgin (ca. 50 mg) was weighed accurately and dissolved in and diluted to 50 ml with ethanol. The solution (5 ml) was
diluted further to 50 ml with the same solvent and the dilute solution (5.0 ml) was analyzed as described under 2.2.5.1 (Table II).

2.2.5.3 **Analysis of analgin tablets**

Twenty tablets were weighed and powdered. The powder equivalent to analgin (ca. 50 mg) was weighed accurately and extracted with 3 x 10 ml ethanol. Each extract was filtered through Whatman No. 40 filter paper and the residue was washed with ethanol. The filtrate and washing were combined in a 50-ml volumetric flask and diluted to the mark with ethanol. The solution (5 ml) was diluted further to 50 ml with the same solvent and the dilute solution (5.0 ml) was analyzed as described under 2.2.5.1 (Table III).

2.2.5.4 **Analysis of analgin injection**

An accurately measured volume of injection was diluted suitably with ethanol to contain analgin equivalent to 100 mcg/ml. An aliquot (5.0 ml) was analyzed as described under 2.2.5.1.
2.2.6.1 Hydrolysis of Analgin in Simulated Gastric Juice

Analgin (125 mg) was weighed accurately and transferred to a 50-ml volumetric flask. It was dissolved in and diluted to 50 ml with simulated gastric juice (at 37°C). The reaction mixture was maintained at 37°C. The sample solution (1 ml) was withdrawn at 2, 3, 4, 5, 6, 7, 10, 15, 20, 30 and 45 minutes and was diluted to 25 ml with absolute ethanol. The dilute solution (0.0 ml) was treated with reagent solution (5 ml) and analyzed as described under 2.2.5.1 (Fig.12).

2.2.6.2 TLC of the hydrolysate of Analgin in Gastric Juice

Sample solutions

Analgin (125 mg) was weighed accurately and transferred to a 50-ml volumetric flask. It was dissolved in and diluted to 50 ml with simulated gastric juice (at 37°C). The reaction mixture was maintained at 37°C. The sample solution (1 ml) was withdrawn at 2, 3, 4, 5, 6, 7, 10, 15, 20, 30 and 45 minutes and was diluted to 25 ml with absolute ethanol.

Procedure

TLC of the hydrolysate was carried out as described under 2.2.4.8 (Fig.13).
2.2.6.3 Effect of concentration of Analgin on rate of hydrolysis

Analgin solutions with different concentration (1 to 5 mg/ml) were prepared in simulated gastric juice and allowed to stand at 37°C. The sample solution was withdrawn at 0, 5, 10, 15, 20 and 60 minutes and chromatographed as described under 2.2.4.8 (Fig. 14).

2.2.7.1 Synthesis of 3,5-diacetyl-1,4-dihydrolutidine according to Nash.

Ammonium acetate (38g, 1M) was mixed with acetylacetone (10g; 0.2M), formaldehyde solution (3.75 ml, 40%) and dissolved in and diluted with water to 500 ml. The solution was allowed to stand for 4 days at 25°C and the precipitates were filtered and dried. It was recrystallized from ethanol. M.P. 195-200°C (reported M.P. 198°C).
2.3 Results and Discussion

In aqueous solution, analgin exists in equilibrium as shown below:

\[ \text{HCHO} + \text{NaHSO}_3 \rightarrow \text{analgin + reagent} \]

USSR Pharmacopoeia describes an identification test for sulphurdioxide and formaldehyde, liberated from analgin on hydrolysis in aqueous acidic solution. Wagner studied the decomposition products of analgin using paper chromatographic and ionophoresis technique (Scheme VI).

Comparison of visible spectra of the reaction mixture containing pure analgin and reagent with that of formaldehyde and reagent shows similar characteristic absorbance spectra having maximum at 412 nm (Fig.15). The standard curve obtained using formaldehyde and that of analgin are shown in Fig.3. It is evident from the data that the analgin hydrolyzes completely under the experimental conditions. Further, this is confirmed by
comparision of calculated molar absorptivity obtained
for analgin with that of formaldehyde reported earlier. In both these cases, it is $8 \times 10^3$ L mol$^{-1}$ cm$^{-1}$.

It is evident (Fig. 4) that in the hydrolysis of analgin maximum formaldehyde is formed at pH 1. It is known that analgin in acidic medium hydrolyses to give 4-hydroxymethylmethylaminoantipyrine (17) and sodium bisulphite. In second step, 4-hydroxymethylmethylaminoantipyrine (17) is decomposed to give formaldehyde and 4-methylaminoantipyrine (18). The compound (17) reacts with (18) to give bismethylantipyrinylmethane (20). In the present study, equivalent amount of formaldehyde is obtained from analgin hydrolysis at pH 1. Thus, it appears that formation of bis compound (20) does not take place at pH 1. Further, the hydrolysed mixture is analyzed by TLC (Fig. 11) which shows that analgin is hydrolyzed to give 4-methylaminoantipyrine (18).

Spectrophotometric procedures for the estimation of analgin based on the reaction between the formaldehyde formed in the hydrolysis of analgin with chromotropic acid$^{59}$ and Schiff reagent$^{60}$ have been reported earlier. The chromotropic acid method does not give reproducible results, while Schiff reagent itself is known to be unstable.
In the present work, analgin is hydrolyzed to form formaldehyde which is then reacted with acetylacetone in presence of ammonium acetate to afford yellow colored products. The reaction is described earlier by Nash.\textsuperscript{18} The reaction conditions are standardized to establish a procedure for the determination of analgin.

In the proposed method, analgin is hydrolyzed at different pH (Fig.4). The formaldehyde formed was reacted with acetylacetone in presence of ammonium acetate at 100\textdegree C. The yellow colored product formed showed maximum absorbance at 412nm (Fig.1). The color intensity reached maximum after keeping the reaction mixture in boiling water bath for 10 minutes (Fig.9) and remained stable for more than 5 hours when kept at room temperature in diffused sunlight. It is stable for more than 24 hours if kept in dark (Fig.10). The Lambert-Beer's law was obeyed in the concentration range of 4 to 24 mcg of analgin per ml of the reaction mixture (Fig.2). The optimum concentration range for the determination of analgin as evaluated from Ringbom plot\textsuperscript{83} was found to be 10 to 24 mcg of analgin per ml. The effective molar absorptivity in terms of analgin was found to be $8.0 \times 10^3$ L mol$^{-1}$ cm$^{-1}$ and the photometric sensitivity\textsuperscript{84} of the color reaction was found to be 0.044 mcg of analgin cm$^{-2}$ at 412nm.
The visible spectrum of reaction mixture containing standard analgin solution (5.0 ml), buffer solution (pH 1; 5 ml) and reagent solution (5 ml) shows characteristics similar to those of pure 3,5-diacetyl-1,4-dihydrolutidine (Fig.15). It is evident from the above observation that 3,5-diacetyl-1,4-dihydrolutidine is formed in the reaction mixture which is a chromophore.

\[
\begin{align*}
\text{HCHO} + \text{NaHSO}_3 & \rightarrow \text{HCHO} + \text{NaHSO}_3 \\
\text{H}_3\text{C}-\text{NCH}_2\text{SO}_3\text{Na} & \rightarrow \text{H}_3\text{C}-\text{NH} + \text{HCHO} + \text{NaHSO}_3
\end{align*}
\]

Pure samples of analgin were analysed by the proposed method, as well as by iodometric method. The results by both the methods are in good agreement (Table I). The purity of the samples of the bulk powder of analgin by the proposed method was 100.19 ± 0.528(n=9) comparing favourably with the official method (Table I).
The proposed procedure was applied to assay analgin alone and in combination with oxyphenbutazone, paracetamol, caffeine, dexamethasone, phenylbutazone, diazepam, papaverin hydrochloride and atropine methonitrate in synthetic mixtures (Table II) as well as in pharmaceutical dosage forms (Table III). None of the combination drugs and the usual diluents, lubricants and solvents employed in formulation of dosage forms interfered in the analysis of analgin by the proposed method. The results are in good agreement with those obtained by the iodometric method or to the labelled amounts of the drug (Table II & III). The method is rapid, accurate and precise.

Hydrolysis of analgin is studied in presence of simulated gastric juice. Analgin is hydrolyzed completely in gastric juice within 10 minutes at 37°C (Fig.12). TLC analysis of reaction mixture shows disappearance of spot corresponding to analgin with increasing time of hydrolysis (Fig.13). Further, it is noteworthy that concentration of analgin influences the rate of hydrolysis (Fig.14). One of the product of hydrolysis is found to be 4-methylamino-antipyrine.
It is evident from the above study that when analgin is given orally, it hydrolyses to give 4-methylaminoantipyrine in stomach. Thus, analgin may be considered prodrug for 4-methylaminoantipyrine.
Figure 1: Visible spectrum of the colored product obtained on reacting analgin with reagent.
Figure 2: Lambert-Beer's curve for Analgin.
Figure 3: Lambert-Beer's curve for formaldehyde.
Figure 4: Effect of pH on the hydrolysis of Analgin.
Figure 5: Effect of buffer (pH 1) concentration on the hydrolysis of Analgin.
Figure 6: Effect of time on hydrolysis of Analgin in buffer (pH 1).
Figure 7: Effect of reagent concentration on the color intensity.
Figure 8: Effect of temperature on color intensity.
Figure 9: Effect of time of heating on color intensity.
Figure 10: Effect of time on stability of colored product
Solvent System: Ethanol : Chloroform : Benzene : 
10% Ammonia solution (5:3:1.8:0.2)

Locating Reagent: Iodine vapours.

Spotting:  
1 = Analgin; 2 = 4-methylaminoantipyrine;  
3 = pH1; 4 = pH2; 5 = pH3; 6 = pH4;  
7 = pH5; 8 = pH6; 9 = pH7; 10 = pH8.

Figure 11: TLC of Analgin Hydrolysate.
Figure 12: In vitro hydrolysis of Analgin.
Solvent System: Ethanol : Chloroform : Benzene :
10% Ammonia Solution (5:3:1.8:0.2)

Locating Reagent: Iodine vapours

Spotting: 1 = 4-Methylaminoantipyrine; 2 = Analgin;
3 = 2 min; 4 = 3 min; 5 = 4 min; 6 = 5 min;
7 = 6 min; 8 = 7 min; 9 = 10 min; 10 = 15 min;
11 = 20 min; 12 = 30 min; 13 = 45 min.

Figure 13: TLC of *in vitro* Analgin Hydrolysate
Solvent System:
Ethanol : Chloroform : Benzene : Ammonia Solution (5:3:1.8:0.2)
Locating Reagent : Iodine vapours
Spotting : 1 = Analgin; 2 = 4-Methylaminoantipyrine; 3 = 0 min;
4 = 5 min; 5 = 10 min; 6 = 15 min;
7 = 20 min; 8 = 60 min.

Figure 14 : Effect of concentration of Analgin on rate of hydrolysis.
Figure 15: Comparison of visible spectrum of Analgin as well as formaldehyde, and 3,5-diacetyl-1,4-dihydrolutidine.


## TABLE I

Analysis of Analgin

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>% Recovery by Proposed Method</th>
<th>Official method $^{34}$</th>
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<tr>
<td>1</td>
<td>100.33</td>
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<td>RSD</td>
<td>± 0.527%</td>
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</table>
## TABLE II

**Analysis of analgin in combination with other drugs in synthetic mixtures**

<table>
<thead>
<tr>
<th>No.</th>
<th>Combination drug (mg)</th>
<th>Analgin (mg)</th>
<th>% Recovery&lt;sup&gt;a&lt;/sup&gt; by Proposed method</th>
<th>% Recovery&lt;sup&gt;a&lt;/sup&gt; by Official method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Oxyphenylbutazone</td>
<td>50</td>
<td>99.98</td>
<td>99.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Paracetamol</td>
<td>100</td>
<td>100.25</td>
<td>100.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>99.96</td>
<td>100.13</td>
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<tr>
<td>3.</td>
<td>Caffeine</td>
<td>10</td>
<td>100.10</td>
<td>99.98</td>
</tr>
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<td></td>
<td>25</td>
<td>100.17</td>
<td>100.05</td>
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<td>4.</td>
<td>Dexamethasone</td>
<td>0.100</td>
<td>99.26</td>
<td>100.26</td>
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<tr>
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<td></td>
<td>0.200</td>
<td>100.76</td>
<td>100.73</td>
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<td>5.</td>
<td>Phenylbutazone</td>
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<td>99.98</td>
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<td></td>
<td>150</td>
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<td>6.</td>
<td>Diazepam</td>
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<td>5</td>
<td>99.76</td>
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<td>7.</td>
<td>Papaverine HCl</td>
<td>10</td>
<td>100.05</td>
<td>100.02</td>
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<td></td>
<td>15</td>
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<td>100.17</td>
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<td>8.</td>
<td>Atropine methonitrate</td>
<td>1</td>
<td>100.20</td>
<td>100.32</td>
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<sup>a</sup> Average value of five determinations.
<table>
<thead>
<tr>
<th>No.</th>
<th>Formulation</th>
<th>Labelled Amount (in mg)</th>
<th>% Recovery&lt;sup&gt;a&lt;/sup&gt; by Proposed method</th>
<th>% Recovery&lt;sup&gt;a&lt;/sup&gt; by Official method&lt;sup&gt;34&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>1</td>
<td>Powder</td>
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<tr>
<td></td>
<td>(i) Analgin powder</td>
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<td>99.92</td>
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<td>(i) Analgin</td>
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<td>(ii) Analgin</td>
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<td>(iii) Analgin</td>
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<td>Paracetamol</td>
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<td>Caffeine</td>
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<td>methonitrate</td>
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<td>97.50</td>
<td>97.40</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average value of five determinations.