CHAPTER - IV

SPECTROPHOTOMETRIC DETERMINATION OF NITROGLYCERIN

4.0 Introduction:

Nitroglycerin is widely used in the treatment of angina pectoris. Various procedures for its estimation are reviewed critically.\(^{169,170}\) They include volumetric, gravimetric, gasometric, UV and visible spectrophotometric, IR spectrophotometric, TLC, GC and HPLC techniques.

I. VOLUMETRIC METHODS:

(i) Acidimetry:

Liquor trinitrin is treated with standard alcoholic sodium hydroxide. The excess of alkali is titrated with standard alcoholic acid using phenolphthalein as indicator.\(^{171}\) Nitroglycerin is saponified with 0.5N sodium hydroxide and excess of sodium hydroxide is titrated against 0.1N hydrochloric acid.\(^{172}\)

It is also titrated as acid in dimethylformamide medium.\(^{173}\)

In absence of alcohol insoluble nitrogenous compounds, nitroglycerin in drug formulations can be assayed by extraction of the dosage forms with ethanol.\(^{174}\) The ethanolic extract is analyzed volumetrically as described earlier.\(^{175}\) If alcohol
soluble nitrogenous compound is present, the nitroglycerin
is extracted with ether and the drug is determined in ethereal
extract. 175

(ii) Kjeldahl's method:

Nitroglycerin is treated with hydrogen peroxide and potassium.
hydroxide. The reaction mixture is reduced with Devarda's
alloy. The ammonia formed is distilled and measured as
usual. 176, 180 The procedure is applied to analyse nitro-
glycerin tablets, 181, 182 and its alcoholic solution. 183, 184
In a variant of this method, the distillation of ammonia is
carried out with alcohol instead of water and the ammonia
is absorbed in ice water instead of acid. 171

A modification in French Pharmacopoeial method is
described in which trinitrin is treated with sodium hydroxide,
ferrous sulphate and zinc powder. The ammonia formed is
distilled and titrated as usual. 185

Nitroglycerin is extracted from tablets with chloroform
The solvent was evaporated. The residue is hydrolyzed with sodium
carbonate solution at 65-70° and ammonia is determined volumetri-
cally. 186

Nitroglycerin is reduced with aluminium and alkali.
The ammonia formed is distilled and estimated as usual. 187-188
The method is applied to assay nitroglycerin tablets.
In micro-kjeldahl's method for determination of nitroglycerin, reduction of nitro group with Adam's catalyst is found to be inadequate, while Devarda's alloy gives good results. The ammonia evolved is absorbed in NaH₂PO₄ and titrated potentiometrically or using bromocresol green as indicator.¹⁰⁹

Nitroglycerin is reduced with Raney nickel in alkaline medium. The ammonia formed is distilled and determined by titration with 0.1N HCl.¹⁰⁰

Nitroglycerin from pharmaceutical preparations is extracted with ether and oxidized with potassium permanganate. The reaction mixture is distilled in presence of Devarda's alloy. The distillate is collected in excess of standard sulfuric acid and back titrated with standard alkali.¹⁷⁵,¹⁹¹ The method is applied to estimate nitroglycerin in dosage forms.¹⁹²,¹⁹³

In another procedure, the ethereal extract of nitroglycerin is refluxed with Devarda's alloy, aluminium wire and alcoholic potassium hydroxide without prior oxidation with KMnO₄. The reaction mixture is distilled. The distillate is analysed for ammonia content as usual. The method gives
somewhat higher results but it is more rapid and convenient than that involving oxidation with potassium permanganate prior to reduction.  

(iii) **Reduction method:**

**Reduction with FeSO₄:**

Nitroglycerin is boiled with ferrous sulfate and sulfuric acid, and cooled. The reaction mixture is titrated against standard ceric sulfate using ferroin as indicator.

Ferric ion produced in the reduction of trinitroglycerin with ferrous sulfate is titrated with EDTA potentiometrically. Solution of nitroglycerin in concentrated H₂SO₄ is directly titrated with ferrous sulfate solution potentiometrically.

An assay procedure for nitroglycerin is reported in which the drug is titrated in sulphuric acid medium with standard ferrous ammonium sulphate solution until brown color appears. Nitroglycerin dissolved in acetic acid is reacted with ferrous chloride. The ferric chloride formed is titrated against standard titanous chloride solution using ammonium thiocyanate as indicator. 2,4-Dinitrotoluene, diethyl-diphenylurea or diphenylamine do not interfere in the determination.
Nitroglycerin is determined by titrimetric method based on their reduction with Fe(OH)$_2$ using diaminesilver sulfate as catalyst followed by titration of Fe$^{+3}$ salt formed with HgNO$_3$.199

**Reduction with titanous chloride:**
Nitroglycerin is reduced with titanous chloride and the excess of titanous chloride is titrated with ferric ammonium sulphate using ammonium thiocyanate as indicator. None of the stabilizers or certain moderants interfere the titration.200,201

**Iodometric method:**
Nitroglycerin after hydrolysis with NaOH reacts with potassium iodide. The liberated iodine is titrated against standard sodium thiosulfate solution.202

Nitroglycerin in pharmaceutical dosage forms was determined by alkali hydrolysis with heating. The precision of the method is increased by acidifying the reaction mixture with H$_2$SO$_4$ to pH 0.1 to 0.5, after hydrolysis. It is then treated with potassium iodide and titrated with standard sodium thiosulfate solution.203
The saponification methods give erratic results.\textsuperscript{182,183} while the reduction method with Davarda's alloy gives lower results.\textsuperscript{183}

II. GRAVIMETRIC:

Nitroglycerin is separated from nitro-body containing explosive by extracting the mixture with ether. The residue of the ethereal extract is dissolved in methanol, acidified with HCl and treated with standard ferric chloride solution. The resulting reaction mixture is extracted with ether. The ethereal extract is washed with water, dried over anhydrous calcium chloride and evaporated. The residue is dried in vacuum, followed by drying over sulphuric acid and weighed.\textsuperscript{204}

III. GASOMETRIC:

Liquor trinitrin is treated with sodium hydroxide and reacted with potassium iodide in presence of sulphuric acid. The gas evolved is measured in a nitrometer.\textsuperscript{205}

IV. SPECTROPHOTOMETRIC METHODS:

Aqueous nitroglycerin solution is mixed 0.1N sodium hydroxide solution and analyzed spectrophotometrically at 331 nm.\textsuperscript{206}
In alkaline solution, nitroglycerin degrades via chromophore intermediate which exhibits an absorption peak at 336 nm. The maximum absorbance reached in 33% water in methanol and 0.033M sodium hydroxide solution.\(^207,208\)

V. **COLORIMETRIC METHODS:**

(a) **Methods based on nitrate ion formation:**

Alcoholic solution of trinitroglycerin is treated with reagent containing sulfuric and phenol or salicylic acid and made alkaline with ammonia solution. The yellow color formed is compared with that obtained in reaction of potassium nitrate with the reagent.\(^183\) Nitrate ion interferes in the method.\(^183\)

Glyceryl trinitrate in tablets is estimated by colorimetric method by reacting nitrate ion formed by hydrolysis of glyceryl trinitrate with phenoldisulphonic acid.\(^188,209\)

A column chromatographic method is proposed for the determination of trinitroglycerin in soluble sublingual tablets. A mixture of phenoldisulphonic acid reagent and acetic acid is used to react with trinitroglycerin and the color of nitrated product is measured.\(^210\)
A method involving nitration of metaxylene with aliphatic nitrates in sulphuric acid is described, the color intensity of reaction product, is measured at 447 μm. The method is applied to assay dosage forms like tablets, ointment, etc. 211

The trinitrin containing formulation is treated with glacial acetic acid and filtered. The filtrate is treated with diphenylamine in concentrated sulfuric acid. The color produced is compared with that produced by reacting potassium nitrate with the reagents. 212 Nitrate ions and other oxidising agents interfere in the procedure.

(b) Methods based on nitrite ion:

The estimation of glyceryl trinitrate is carried out in presence of pentaerythritol tetranitrate in tablet. Glyceryl trinitrate hydrolyzes by 0.1N sodium hydroxide in 1 hrs. at 20°C, while pentaerythritol tetranitrate remains unaffected. The liberated sodium nitrite diazotizes sulphanilic acid in presence of hydrochloric acid. The diazotized sulphanilic acid is coupled with 1-naphthylamine and the color produced is measured at 530nm. 213

Glyceryl trinitrate in pellets is determined spectrophotometrically. The method is based on hydrolysis of the drugs by strontium hydroxide. The nitrite formed diazotizes procaine, followed by coupling with naphthylethylenediamine. 214
Nitroglycerin in blood and urine is extracted with ether, hydrolyzed with alcoholic potassium hydroxide. The potassium nitrite produced is estimated colorimetrically at 490 nm after diazotisation of p-nitroaniline and coupling with naphthylamine.\(^\text{215}\)

A spectrophotometric procedure for micro-determination of glyceryl trinitrate based on alkaline hydrolysis followed by determination of the nitrite, formed through diazotisation and coupling is described. Interference by lactose is avoided by using strontium hydroxide in place of sodium hydroxide as the hydrolyzing alkali.\(^\text{216}\)

Nitroglycerin is hydrolyzed to nitrite, which diazotises p-chloroaniline. The diazonium salt is coupled with N-1-naphthyl)ethylenediamine dihydrochloride and the color formed is measured at 560 nm.\(^\text{217}\) The method is applied to assay nitroglycerin tablets.

Nitroglycerin in air is dissolved in ethanol and the solvent is evaporated on a steam bath. The residue is treated with 3N Potassium hydroxide and evaporated to dryness. Every molecule of nitroglycerin gives 2 molecule of potassium nitrite and 1 molecule of potassium nitrate. The nitrite ion is determined colorimetrically.\(^\text{218}\)
Nitroglycerin is hydrolyzed in aq. alkali. The nitrile formed diazotises procaine which is then coupled with Bratton-Marshall reagent. The absorbance of the resultant color is measured at 550nm.

Trinitroglycerin is determined by alkaline hydrolysis with 0.2M sodium hydroxide solution, reduction with hydrazine hydrate in the presence of Cu$^{2+}$, diazotization and coupling with stable reagent and measurement of the absorbance. Each step of the assay was optimized with regard to operating conditions and adopted for use with an automatic apparatus. The hydrolysis of nitroglycerin occurs by $\alpha$-hydrogen elimination resulting in the formation of nitrite ions. Reduction is necessary step for the color development in the case. High concentration of some sugars interfered with the spectrophotometric determination.

The diazotisation method is preferable to the 2,4-phenol-disulfonic acid method for the determination of nitroglycerin in drug tablets and capsules, since (i) a quantitative extraction is achieved in a simple manner, (ii) there is little interference from other substances, (iii) the sensitivity is about 6-fold higher.
(c) **Other Colorimetric methods:**

A method for determination of nitroglycerin in sublingual and oral tablet is reported. The method depends on the extraction of the drug with glacial acetic acid, absorption on celite and elution with iso-octane. The eluate is treated with tetramethylammonium hydroxide in the presence of p-chloroaniline in propanol and the color formed is measured.²⁰⁸,²²²

The aqueous extract of nitroglycerin tablet was treated with ethacridine lactate to form a diazo derivative. The color formed is measured at 490nm.²²³

The aqueous extract of nitroglycerin tablet is hydrolyzed with sodium hydroxide. The resultant sodium nitrite is treated with proflavine or flavacridine hydrochloride. The excess of diaminoacridine is measured at 540nm.²²⁴

Nitroglycerin is treated with conc. sulfuric acid and acetone. The blue color in acetone layer is measured colorimetrically. The method is preferred to the earlier reported methods.²²⁵
VI. **IR SPECTROPHOTOMETRIC METHOD**:

The drug is extracted with carbon disulphide from ammonium hydroxide solution. Concentrated and diluted with carbon disulphide. Measurements at 9.66 micron gives quantitative recovery and offers quantitative proof of identity. \(^{226,227}\)

IR Spectrophotometric method is applied to determine nitroglycerin in propellant mixtures. \(^{228}\)

Glyceryl trinitrate is determined by using the N-O asymmetric stretching vibration bond. Chloroform and tetrahydrofuran were used as solvents. \(^{229}\)

VII. **POLAROGRAPHIC**:

A polarographic method for the determination of glyceryl trinitrate in tablets is described. \(^{230}\) Polargraphic procedure of nitroglycerin in non-aqueous medium is reported. \(^{231}\)

Stationary silver electrodes were used for polarographic determination of nitroglycerin in solution. \(^{232}\)

A polarographic assay of glyceryl trinitrate using methyl nitrate as internal standard is described. \(^{233}\)
VIII THIN LAYER CHROMATOGRAPHIC:

Nitroglycerin is separated from other propellants by TLC using butanol : acetic acid : water (4:1:1) as solvent system. The spots were developed by spraying the plate with alcoholic solution of diphenylamine and green spot was observed under UV light. The technique is also employed for quantitative determination.\(^{234,235}\)

Nitroglycerin was determined in ointment by extraction with toluene-hexane (1:1) mixture and chromatographed on silica gel plates and developed with the same solvent as mobile phase. Reflectance was measured at 209 nm.\(^{236}\)

Thin layer chromatographic separation of nitroglycerin is carried out on silica gel plates and the developed spot is measured by color densitometer.\(^{237}\)

IX GAS CHROMATOGRAPHY:

A gas chromatograph with a thermal conductivity detector was used to assay glyceryl trinitrate.\(^{238}\) A gas chromatographic technique is applied for determination of nitroglycerin in various pharmaceutical formulations.\(^{239}\)
A specific assay for nitroglycerin in dosage form using HPLC is developed.\textsuperscript{240,241}

Ointment of nitroglycerin is dissolved in warm methyl alcohol and analyzed by HPLC.\textsuperscript{242}

A high performance liquid chromatographic assay for partially nitrated glycerin in nitroglycerin is described.\textsuperscript{243} Sublingual, sustained release tablet and ointment of nitroglycerin are dissolved in methanol and filtered. The filtrate was analysed for nitroglycerin content by HPLC.\textsuperscript{244} HPLC with spectrophotometric detection was used for the determination of nitroglycerin using methanol-water (1:1) mixture as solvent.\textsuperscript{245}

Nitroglycerin and its degradation products were analyzed by HPLC with a column packed with 34 μm diameter spherical silica with octadecylsilane bonded phase and a mobile phase of methanol–acetonitrile–water (24:24:52).\textsuperscript{246}

In Chapter-2, a spectrophotometric method for the determination of nitrite ion in micro-quantities is described. It was, therefore, thought of interest to analyse nitroglycerin by the same method after its hydrolysis.
In the present work, nitroglycerin is hydrolyzed in presence of potassium hydroxide. Procaine hydrochloride is diazotized with the liberated nitrite as hydrolysis product. The diazonium salt is coupled with N-1-(naphthyl)-ethylenediaminedihydrochloride to obtain pink colored solution having maximum absorbance at 545nm. Various reaction conditions are standardized to obtain maximum color intensity. The Lambert-Beer's law is obeyed in the concentration range of 0.1 mcg to 1.6 mcg per ml of reaction mixture. The method is applied to assay nitroglycerin and its dosage forms. The results are in good agreement with those obtained by pharmacopeial method. The procedure is successfully employed to determine nitroglycerin in blood and urine.
4.1 Experimental:

Apparatus:
1. Double beam Hitachi Spectrophotometer, Model 220S, having two matched cells of 1 cm light path.
2. Constant temperature water bath (Townson and Mercer Ltd., England).
3. Corning volumetric flasks of 25, 100 and 500ml capacity.

Reagents and Materials:
Procaine hydrochloride BP, Hydrochloric acid BP, N-(naphthyl)ethylenediamine dihydrochloride (GR), Potassium Hydroxide (Pellets, BDH) and double distilled water were used in the study.

Preparation of Procaine hydrochloride solution: (0.5% w/v)
Procaine hydrochloride (500mg) was dissolved in and diluted to 100ml with water.

Preparation of N-1-(naphthyl)ethylenediamine dihydrochloride solution (0.1% w/v):
N-1-(Naphthyl)ethylenediamine dihydrochloride (100mg) was dissolved in and diluted to 100ml with water.
Preparation of 0.1M Potassium hydroxide solution:

Potassium hydroxide (0.56g) was dissolved in and diluted to 100ml with water.

Preparation of Standard Nitroglycerin solution:

Nitroglycerin (50mg) 10% w/w was weighed accurately and dissolved in and diluted to 500ml with water. Each ml. contains 10 mcg.

Preparation of 4M Hydrochloric acid solution:

Concentrated hydrochloric acid to (39.5 ml) was diluted to 100ml with distilled water, and standardised against anhydrous sodium carbonate.

Preparation of Phenoldisulphonic acid solution:

Phenol (3.0g) was heated with sulphuric acid (20.0ml) on a boiling water bath for six hours. The resulting liquid was stored in a stoppered bottle.

PROCEDURE:

4.1. Effect of Concentration of Nitroglycerin:

Standard nitroglycerin solution (0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0ml) was pipetted into series of 25ml volumetric flasks and diluted to 10ml with water. Potassium hydroxide solution (1.0ml, 0.1M) was added to it.
and mixed thoroughly. The flask was immersed in a boiling water bath for 30 min and then cooled to room temperature. Procaine hydrochloride (1.0ml, 0.5% w/v) was added to it and was allowed to stand for 3 min at room temperature. Hydrochloric acid (1.0ml, 4M) was added to it and mixed thoroughly. N-1-(naphthyl)ethylenediamine dihydrochloride solution (1.0ml) was added to the reaction mixture and was allowed to stand at room temperature for 10 min. The volume was adjusted to the mark with water. After 30 min, the absorbance was measured at 545nm against sample blank. The sample blank was prepared by omitting alkali addition (fig.1).

4.1.1 Analysis of Nitroglycerin:

Nitroglycerin (50 mg) was weighed accurately and dissolved in and diluted to 500ml with water. Solution (2.0ml) was pipetted into 25ml volumetric flask and diluted to 10.0ml with water, and treated as descibed under 4.1. The amount of nitroglycerin was determined by referring to the standard curve (Table-I).

4.1.2 Analysis of Nitroglycerin Tablets:

Twenty tablets were weighed and powdered. The powder equivalent to nitroglycerine (ca 2.5mg) was weighed accurately and transferred into 250ml volumetric flask and dissolved in
water (200ml) by shaking on shaker for one hour. It was then diluted to 250 ml with distilled water and filtered. The filtrate (2ml) was analysed as described under 4.1 (Table-II).

4.1.3 Analysis of nitroglycerin ointments:

The ointment equivalent to nitroglycerin (ca 5.0mg) was weighed accurately on piece of butter paper and transferred to 500ml volumetric flask containing 25ml of ethanol. The sample was dispersed uniformly by shaking and slightly warming on water bath. It was then diluted to the mark with water. The reaction mixture (2ml) was analysed as described under 4.1 (Table-II).

4.1.4 Analysis of (sustained released) nitroglycerin capsules:

Contents of twenty capsules were mixed and powdered. The powder equivalent to nitroglycerin (ca 2.5mg) was weighed accurately and transferred to 250ml volumetric flask containing acetone (20ml). The sample was dissolved by shaking and slightly warming on water bath. It was then diluted to the mark with water. The solution (2ml) was analysed as described under 4.1 (Table-II).
4.1.5 Analysis of nitroglycerin by I.P. procedure:

Accurately weighed nitroglycerin (100mg) was transferred to 100 volumetric flask and mixed with acetic acid 70.0ml; 90% v/v. The reaction mixture was shaken for 15 minutes and diluted to 100ml volume with acetic acid (90% v/v) and centrifuged. The supernatent liquid (2.0ml) was mixed with phenol disulphonic acid solution (2ml) and allowed to stand for 15 minutes. After adding 8ml of water, it was made alkaline with strong ammonia solution and cooled to about 20°. It was then diluted to 25.0ml with water and filtered. The absorbance of the filtrate was measured at 405nm using acetic acid (2.0ml; 90% v/v), treated similarly, as blank.

Potassium nitrate (133.5mg) previously dried, was dissolved in and diluted to 100ml with water. The solution (10ml) was diluted to 100ml with glacial acetic acid. The dilute solution (2ml) was mixed with phenol disulphonic acid solution (2ml) and was allowed to stand for 15 minutes. After mixing with water (8.0ml), it was made alkaline with strong ammonia solution, cooled to about 20°. It was diluted to 25ml with water and filtered. The absorbance of the filtrate was measured at 405nm, using acetic acid (2.0ml; 90% v/v) treated similarly as reagent blank (Table-II).
4.1.6 Analysis of nitroglycerin in presence of sodium nitrite

by proposed procedure:

Sodium nitrite (50mcg, or 100mcg) was placed in 100ml volumetric flasks. Standard nitroglycerin solution (10ml;100mcg/ml) was pipetted into it and mixed thoroughly. The reaction mixture was diluted to the mark with water. An aliquot of solution (2ml) was analysed as described under 4.1 (Table-III).

4.1.7 Analysis of nitroglycerin in presence of sodium nitrite

by I.P. procedure:

Nitroglycerin (500mg) was weighed and transferred to the 100ml volumetric flask, containing 70ml of 90% v/v acetic acid. It was shaken for 15 minutes and diluted to mark with 90% v/v acetic acid. The reaction mixture was centrifuged. The supernatant solution (5ml) was transferred to 25ml volumetric flask containing sodium nitrite (50mcg or 100mcg) in 90% v/v acetic acid, and diluted to mark with 90% v/v acetic acid and mixed. An aliquot of solution (2ml) was mixed with phenol disulphonic acid solution (2.0ml) and allowed to stand for 15 minutes. It was further analysed by procedure described under 4.1.5. (Table III).

4.1.8 Analysis of nitroglycerin in presence of sodium nitrate

by proposed procedure:

Standard nitroglycerin solution (5ml) was mixed with sodium nitrate (50mcg, or 100mcg) in a 100ml volumetric flask.
It was then diluted to 100ml with water. The resulting solution (2ml) was analysed as described under 4.1 (Table-III).

4.1.9 Analysis of nitroglycerin in presence of sodium nitrate by I.P. procedure:

Nitroglycerin (500mg) was weighed and transferred to the 100ml volumetric flask, containing 70ml of 90% v/v acetic acid. It was shaken for 15 minutes and diluted to mark with 90% v/v acetic acid and centrifuged. The supernatant liquor (5ml) was transferred to 25ml volumetric flask, containing (50mcg or 100mcg) of sodium nitrate in 90% v/v acetic acid, and diluted to mark with 90% v/v acetic acid and mixed. An aliquot of solution (2ml) was analysed by procedure described under 4.1.7. (Table III).

4.1.10 Analysis of nitroglycerin in presence of isosorbide-dinitrate by proposed procedure:

Nitroglycerin (100mg) and isosorbidedinitrate (25mg) were weighed accurately and suspended in water (60ml). The suspension was shaken for 15 minutes and diluted to 100ml with water. The reaction mixture was filtered through Whatman No.41 filter paper. The filtrate (10ml) was diluted to 100ml with water. An aliquot of the solution (2ml) was analysed by procedure described under 4.1 (Table-IV).
4.1.11 Analysis of nitroglycerin in presence of isosorbidentinitrate by 1,P. procedure:

Accurately weighed nitroglycerin (100mg) and isosorbidentinitrate (40mg) were transferred to 100ml volumetric flask, containing 70.0ml of acetic acid 90% v/v. The reaction mixture was shaken for 15 minutes and diluted to 100ml volume with acetic acid (90% v/v) and centrifuged. The supernatant liquid (2.0ml) was mixed with phenol disulphonic acid solution (2.0ml) and allowed to stand for 15 minutes. It was further analysed by procedure described under 4.1.7. (Table IV).

4.1.12 Analysis of nitroglycerin in presence of pentaerythritol tetranitate by proposed procedure:

Nitroglycerin (100mg) and pentaerythritol tetranitate (100mg) were weighed accurately and mixed with (60ml) water. The reaction mixture was shaken for 15 minutes and diluted to 100ml with water. It was then filtered through Whatman No.41 paper. The filtrate (10ml) was diluted further to 100ml with water. An aliquot of the solution (2ml) was analysed by procedure described under 4.1 (Table-IV).
4.1.13 Analysis of nitroglycerin in presence of pentaerythritol tetranitrate by I.P. procedure:

Accurately weighed nitroglycerin (100mg) and (100mg) pentaerythritol tetranitrate were transferred to 100ml volumetric flask, containing 70.0ml of acetic acid (90% v/v). The reaction mixture was shaken for 15 minutes and diluted to 100ml with acetic acid (90% v/v) and centrifuged. The supernatant liquid (2.0ml) was mixed with phenol disulphonic acid solution (2.0ml) and allowed to stand for 15 minutes. It was analysed by procedure described under 4.1.7. (Table I).

4.1.14 Analysis of nitroglycerin in urine using acetone as solvent by proposed procedure:

Nitroglycerin (100mg) was weighed accurately and dissolved in urine (5ml) in a 50ml beaker, and shaken occasionally. After 30 minutes the reaction mixture was transferred quantitatively to 100ml volumetric flask by the aid of acetone and diluted to volume with acetone. The reaction mixture was filtered through Whatman No.41 filter paper. The filtrate (10ml) was diluted to 100ml with water and mixed.

The aliquot (2ml) was pipetted into 25ml volumetric flask and diluted to 10ml with water, mixed potassium hydroxide (1ml, 0.1M) and analysed as described under 4.1. (Table V).
4.1.15 **Analysis of nitroglycerin in urine by I.P. procedure using acetone as diluent:**

Nitroglycerin (100mg) was weighed accurately and mixed with urine (5ml) in a 50ml beaker and shaken occasionally. After 30 minutes, the reaction mixture was transferred quantitatively to the 100ml volumetric flask, with the aid of acetone and diluted to volume with the same solvent. The reaction mixture was filtered through Whatman No.41 filter paper. The filtrate (2ml) was pipetted into 25ml volumetric flask, mixed phenol disulphonic acid solution (2ml) and analysed as described under 4.1.7. (Table V).

The amount of nitroglycerin was calculated by referring to the standard curve using potassium nitrate as reference standard.

4.1.16 **Analysis of nitroglycerin in urine using water as solvent by proposed procedure:**

Nitroglycerin (100mg) was weighed accurately and dissolved in urine (5ml) in a 50ml beaker, and shaken occasionally. After 30 minutes the reaction mixture was transferred quantitatively to 100ml volumetric flask with the aid of distilled water and diluted to volume with distilled water. The reaction mixture was filtered through Whatman No.41 filter paper. The filtrate
(10ml) was diluted further to 100ml with water. An aliquot of the solution (2ml) was pipetted into 25ml volumetric flasks and diluted to 10ml with water, mixed potassium hydroxide (1ml; 0.1m) and analysed as described under 4.1 (Table V).

The amount of nitroglycerin was calculated by referring to the standard curve using nitroglycerin as reference standard.

**4.1.17 Analysis of nitroglycerin in urine by I.P. procedure using 90% v/v acetic acid as solvent:**

Nitroglycerin (100mg) was weighed accurately and mixed with urine (5ml) in a 50ml beaker and shaken occasionally. After 30 minutes, the reaction mixture was transferred quantitatively to the 100ml volumetric flask with the aid of acetic acid (90% v/v) and diluted to volume with the same solvent. The reaction mixture was filtered through Whatman No.41 filter paper.

The filtrate (2ml) was pipetted into 25ml volumetric flask, mixed with phenol disulphonic acid solution (2ml) and analysed as described under 4.1.7. (Table V).

The amount of nitroglycerin was calculated by referring to standard curve using potassium nitrate as reference standard.
4.1.18 Analysis of nitroglycerin in blood using acetone as solvent by proposed method:
The analysis of nitroglycerin in blood was carried out as described under 4.1.14 replacing urine by blood serum. (Table V).

4.1.19 Analysis of nitroglycerin in blood using water as solvent by proposed method:
The analysis of nitroglycerin in blood was carried out as described under 4.1.16 replacing urine by blood serum. (Table V).

4.1.20 Analysis of nitroglycerin in blood by I.P. procedure using acetone as solvent:
The analysis of nitroglycerin in blood was carried out as described under 4.1.15 replacing urine by blood serum. (Table V).

4.1.21 Analysis of nitroglycerin in blood by I.P. procedure using 90% v/v acetic acid as diluent:
The analysis of nitroglycerin in blood was carried out as described under 4.1.17 replacing urine by blood serum. (Table V).
4.2 Results and Discussion:

Nitroglycerin on hydrolysis under experimental conditions gives nitrite ions (Chapter III). Procaine hydrochloride is diazotised with the liberated nitrite ion and the diazonium salt is coupled with N-1-(naphthyl)ethylenediamine dihydrochloride to give pink colored product having maximum absorbance at 545 nm (Chapter II).

In the present work, a spectrophotometric method for the determination of nitroglycerin based on above reaction, is worked out. The method is applied to assay nitroglycerin in bulk powder, as well as in its dosage form. The results are comparable with those obtained by pharmacopoeial method or the labelled amount (Table I-II). Presence of nitrate pentaerythritol tetranitrate, isosorbid dinitrate diluents, lubricants and other auxiliary materials employed in the preparation of dosage forms does not interfere in the estimation of nitroglycerin by the proposed procedure. In presence of nitrate ions, higher recovery percent are obtained when nitroglycerin is analyzed by pharmacopoeial method (Table III).

The proposed method was applied to determine nitroglycerin in biological fluids (Table V). In the analysis of nitroglycerin in blood and urine, when water was used as diluting solvent, slightly lower recovery of nitroglycerin was obtained. However, satisfactory recovery are obtained when the reaction mixture was diluted with acetone. It is noteworthy that the effect of diluting solvent conspicuous when the drug in blood or urine is estimated by pharmacopoeial method (Table V).
FIG. 1. LAMBERT - BEER'S CURVE
<table>
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<th>Official Method*</th>
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<td>SEM</td>
<td>0.035</td>
<td>0.064</td>
</tr>
<tr>
<td>% CV</td>
<td>1.053</td>
<td>1.913</td>
</tr>
</tbody>
</table>

* Recovery percent

Standard deviation is 0.67 and % CV is 2.0% which shows that the proposed method is precise and reproducible.

SEM : Standard error of the mean $\frac{S.D}{n}$

% CV : % of co-efficient of variation with infra assay
TABLE - II: ANALYSIS OF NITROGLYCERIN DOSAGE FORMS

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Labelled Amount</th>
<th>% Recovery* by Proposed method</th>
<th>% Recovery* by Official method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tablet:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Nitroglycerin</td>
<td>0.5mg/tab.</td>
<td>0.4948</td>
<td>0.4954</td>
</tr>
<tr>
<td>2. Nitroglycerin</td>
<td>0.5mg/tab.</td>
<td>0.4956</td>
<td>0.4906</td>
</tr>
<tr>
<td><strong>Capsules</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Substained release)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Nitroglycerin</td>
<td>2.5mg/cap.</td>
<td>2.613</td>
<td>4.422</td>
</tr>
<tr>
<td>2. Nitroglycerin</td>
<td>6.5mg/cap.</td>
<td>7.157</td>
<td>9.786</td>
</tr>
<tr>
<td>3. Nitroglycerin</td>
<td>9.0mg/cap.</td>
<td>9.751</td>
<td>12.243</td>
</tr>
<tr>
<td><strong>Ointment:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Nitroglycerin</td>
<td>2.0% w/w</td>
<td>1.980%</td>
<td>2.0304%</td>
</tr>
<tr>
<td>2. Nitroglycerin</td>
<td>2.0% w/w</td>
<td>1.9816%</td>
<td>1.9872%</td>
</tr>
</tbody>
</table>

* Average value of five determinations.

**Note:**
1. In time released capsules; overages may be 5 to 10 percent of labelled amount.
2. Results by I.P. method are very high as compared to results by proposed method.
3. No interference in proposed method.
### TABLE-III : ANALYSIS OF NITROGLYCERINE IN PRESENCE OF SODIUM NITRITE AND SODIUM NITRATE:

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Nitroglycerine mg</th>
<th>Sodium Nitrite mg</th>
<th>Sodium Nitrate mg</th>
<th>a) % Recovery by Proposed Method</th>
<th>I.P. Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>-</td>
<td>-</td>
<td>9.991</td>
<td>10.100</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>50</td>
<td>-</td>
<td>9.8200</td>
<td>9.820</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>100</td>
<td>-</td>
<td>9.874</td>
<td>10.042</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>-</td>
<td>50</td>
<td>10.134</td>
<td>10.872</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>-</td>
<td>100</td>
<td>10.073</td>
<td>11.148</td>
</tr>
</tbody>
</table>

a) Average of five determinations

### TABLE - IV : ANALYSIS OF NITROGLYCERINE IN PRESENCE OF PENTAERYTHRITOL TETRANITRATE AND ISOSORBIDE DINITRATE :

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Nitroglycerine mcg</th>
<th>Pentaerythritol tetranitrate mg</th>
<th>Isosorbide dinitrate mcg</th>
<th>a) % Recovery by Proposed Method</th>
<th>I.P. Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>10.126</td>
<td>10.152</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>18</td>
<td>-</td>
<td>10.142</td>
<td>19.780</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>-</td>
<td>20</td>
<td>10.180</td>
<td>17.3032</td>
</tr>
</tbody>
</table>

a) Average of five determinations.
**TABLE - V : ANALYSIS OF NITROGLYCERINE (NG) IN BLOOD AND URINE**

<table>
<thead>
<tr>
<th>Experimental details</th>
<th>Percentage Recovery* By Proposed Method</th>
<th>Percentage Recovery By I.P. Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG + Water</td>
<td>9.98</td>
<td>9.97</td>
</tr>
<tr>
<td>NG + Acetone</td>
<td>9.99</td>
<td>10.01</td>
</tr>
<tr>
<td>NG + Serum + Water</td>
<td>9.50</td>
<td>6.30</td>
</tr>
<tr>
<td>NG + Urine + Water</td>
<td>9.50</td>
<td>6.84</td>
</tr>
<tr>
<td>NG + Serum + Acetone</td>
<td>10.08</td>
<td>11.28</td>
</tr>
<tr>
<td>NG + Urine + Acetone</td>
<td>9.96</td>
<td>11.21</td>
</tr>
</tbody>
</table>

* Average of five determinations.