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Application of Precipitation and Complexation Reactions for the Analysis of Propranolol Hydrochloride

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Two methods, one titrimetic and the other spectrophotometric, for the determination of propranolol hydrochloride in bulk drug and in its tablets have been described. Both methods are indirect and involve the determination of the chloride content of the hydrochloride of the drug. In titrimetry, the sample solution is treated with a known excess of silver nitrate and after the precipitations is complete, unreacted silver nitrate is back titrated with thiocyanate using iron (III) as indicator. Spectrophotometry makes use of the reaction between chloride and mercury (II) thiocyanate in which thiocyanate ions will be displaced and complexed with iron (III) for subsequent measurement at 460 nm. Titrimetry is applicable for 1-9 mg of drug whereas spectrophotometry is applicable in the concentration range of 10-50 μg/ml. The detection limit, the quantification limit, molar absorptivity and Sandell sensitivity values of the spectrophotometric method have been reported. These methods were successfully applied to the determination of propranolol in tablets with the percentage recovery of 97.9-100.9 % (titrimetry) and 99.1-102.5 % (spectrophotometry).

Propranolol belongs to beta-adrenoceptor antagonist class of drugs[1]. It is currently used for treating angina pectoris, hypertension, migraine, anxiety attacks and thyrotoxicosis. A number of methods are available for the determination of propranolol in biological fluids[2-5] while few methods are reported for its assay in pharmaceutical preparations. Official methods described in BP[6] and USP[7] utilise uv-spectrophotometry after extraction into methanol which are non-specific because they are based on the absorbance of the methanolic solution of the tablet extract in the uv region (290 nm). Sensitive methods suggested for propranolol include colorimetry or spectrophotometry[8-17] fluorimetry[18], phosphorimetry[19] and high-performance liquid chromatography[20-22], but the facility required is not always widely available in the last three instances. Spectrophotometric methods reported, though simple and sensitive, suffer from one or the other disadvantages. The nitration method[8] besides involving heating at 80-90° for 20 mm suffers from interference from the decomposition products of nitric acid whereas the nitrosation method[9] uses very high sulphuric acid concentration. The indirect kinetic method[10] is tedious, time-consuming and requires strict adherence to experimental conditions. A derivative of propranolol with 2,4-dinitro-1-fluorobenzene has been employed for its determination by Singhbal and Prabhudesai[11], but the reagent is coloured and therefore, requires strict blank compensation. The spectrophotometric methods based on the use of 2,3-dichloro-1,4-naphthaquinone[12], chloranil-acetaldehyde[13] and cerium (IV)[14] involve heating the reaction mixture for 20-30 min. The chief limitation of the method employing N-bromosuccinimide and celestine blue as reagents[15] is that the latter is never available in pure state and hence, the results are not reliable since the method is based ultimately on the measurement of absorbance of celestine blue. The colour reaction with bromothymol blue[16] and supracen violet 3B[17] involve extraction of the chromogen into organic solvent, a step which is tedious and time-consuming. Only two titrimetric procedures, a.c. oscillopolarographic[23] and conductometric[24] employing sodium tetraphenylborate and ammonium reineckate, respectively, as reagents have been reported for the assay of propranolol.

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This paper describes two simple, accurate and selective methods for the determination of propranolol hydrochloride (PPH) in pure sample and dosage forms. The titrimetric method is based on the precipitation reaction and the spectrophotometric method on the complex formation reaction.

MATERIALS AND METHODS

An Elico model SL-171 digital spectrophotometer with 1-cm glass cells was used for absorbance measurements. All chemicals used were of analytical reagent grade. Double distilled water, second time distilled over alkaline potassium permanganate was used throughout. An approximately 0.04 M silver nitrate solution was prepared by dissolving about 1.7 g of sample (Sarabhai Chemicals, Vadodara) in water and diluting to volume in a 250 ml standard flask. The solution was standardised against sodium chloride[25]. The solution was stored in amber coloured bottle and kept in dark when not in use. Working solutions were prepared by appropriate dilution when required. An approximately 0.02 M potassium thiocyanate solution was prepared by dissolving about 0.8 g of sample (Ranbaxy Chemicals, New Delhi) in water and diluting to 500 ml in a volumetric flask, and standardised by Volhard method[26]. Iron (III) indicator was prepared by dissolving about 10 g of ferric ammonium sulphate in 100 ml of 1:1 nitric acid and boiling the solution till the oxides of nitrogen were expelled. Iron (III) nitrate reagent was prepared by dissolving 15.1 g of sample (BDH) in 45 ml of 72% perchloric acid and diluting to 100 ml with water. This solution was 0.375 M in iron (III) and 5.25 M in perchloric acid. A saturated solution of mercury (II) thiocyanate (Loba Chemie, Mumbai) in methanol and 1:1 nitric acid (S.D. Fine Chem, Boisar) were prepared in the usual way. Chloride - free nitrobenzene was used for titrimetric work.

Pharmaceutical grade PPH was gifted by Cipla India Ltd., and used as received. A stock solution of PPH containing 2 mg/ml was prepared by dissolving 500 mg of sample in water and diluting to 250 ml in a volumetric flask. For spectrophotometric work this solution was diluted stepwise to get a working solution of 100 μg/ml. Twenty tablets were weighed and powdered. The powder equivalent to 500 mg of PPH was treated with 250 ml of water, the insoluble residue followed to obtain a solution of 2 mg/ml. The stock solutions were further diluted to get 100 μg/ml solutions for spectrophotometric work.

Assay procedures:

An aliquot measuring 10 ml of solution containing 1-9 mg PPH was pipetted into a 100 ml Erlenmeyer flask and acidified with 2 ml of 1:1 nitric acid. Then, 5 ml of 0.04 M silver nitrate was introduced by means of a pipette and shaken thoroughly for a min. Finally, 2 ml of nitrobenzene were added and shaken vigorously until the silver chloride was coagulated, 0.5 ml of iron (III) alum indicator were added and the residual silver nitrate was titrated with standard 0.02 M potassium thiocyanate solution to a permanent red colour end-point. A blank was run in the same way. The drug content was calculated using the formula (B-S) MW/n, where B is the volume of thiocyanate consumed in the blank titration, S is the volume of thiocyanate consumed in the test sample titration, M is the molarity of thiocyanate solution, W is the molecular weight of drug and n is the number of moles of silver nitrate reacting with one mole of drug.

Into a series of 10 ml standard flasks were transferred 0, 1, 2, 3 ..... 5 ml of 100 μg/ml of PPH solution by means of a microburette. One millilitre volume of iron (III) nitrate reagent and 2 ml of mercury (II) thiocyanate solution were added and diluted to volume with water. The solution was mixed well and absorbance measured against the reagent blank at 460 nm after 10 mm. The increase in absorbance was plotted against the PPH concentration. The concentration of the unknown was read from the calibration graph or computed from the linear regression equation. Convenient aliquots of tablet solutions (2 mg/ml for titrimetry and 100 μg/ml for spectrophotometry) were subjected to analysis by the above procedures.

RESULTS AND DISCUSSION

Majority of the pharmaceutically important organic compounds are prepared as hydrochlorides and some have been assayed by determining their chloride content[27-32]. No literature reports were found describing the assay of PPH via determination of its chloride content. This communication deals with the determination of PPH by two methods. In titrimetry, the drug was determined by measuring its chloride content by Volhard method[33]. The spectrophotometric procedure involves the reaction of chloride with mercury (II) thiocyanate to form soluble mercury (II) chloride with the liberation of thiocyanate ions which then react with iron (III) to form the familiar red colour which can be measured at 460 nm.

Volhard method has been used for the indirect determination of chloride in diverse matrices including pharmaceuticals[34,35]. In the present method, a known excess of standard silver nitrate solution is added to the drug solution and the excess back titrated with standard thiocyanate solution

using iron (III) as indicator and in the presence of nitrobenzene. PPH in aqueous solution ionizes to give the protonated drug moiety and chloride ion, the latter being reacted with Ag^+ as follows:

$$PPHCl \rightleftharpoons PPH^+ + Cl^-$$

$$Cl^- + Ag^+ \text{ (excess)} \rightleftharpoons AgCl(s) + Ag^+ \text{ (unreacted)}$$

$$Ag^+ \text{ (unreacted)} + SCN^- \rightleftharpoons AgSCN\ (s)$$

Iron (III) serves as the indicator imparting a red colour to the solution to the first excess of thiocyanate ion:

$$Fe^{3+} + SCN^- \rightleftharpoons Fe\ SCN^{2+} \text{ (red)}$$

A 0.5 ml of indicator solution and 2 ml of 1:1 nitric acid in a total volume of about 20 ml gave satisfactory results. AgCl is more soluble than AgSCN and hence leads to low values of chloride analysis and drug recovery. This source of error is circumvented by the addition of 2 ml of nitrobenzene and shaking the reaction mixture thoroughly before the back titration of the residual silver nitrate[36]. The calculated molar ratio of 1:1 between PPH and $AgNO_3$ is consistent with the reaction scheme shown above and was used for calculations.

There are several reports on the spectrophotometric determination of chloride in various matrices[37-41]. One of the most widely used methods for the determination of chloride at low levels consists of spectrophotometric measurement at 460 nm of the coloured iron (III) thiocyanate complex[37-41]. In the proposed method, the dissociated chloride of PPH displaces thiocyanate from mercury (II) thiocyanate and the liberated thiocyanate reacts with iron (III) to form red coloured complex $FeSCN^{2+}$ which is measured at 460 nm. The essential reactions involved are:

$$PP\ HCl \rightleftharpoons PPH^+ + Cl^-$$

$$2Cl^- + H(SCN)_2 \rightleftharpoons HgCl^2 + 2\ SCN^-$$

$$SCN^- + Fe^{3+} \rightleftharpoons FeSCN^{2+} \text{ (red)}$$

The absorbance of the coloured complex measured is a quantitative measure of the concentration of PPH. The wavelength of maximum absorption was found at 460 nm which is in agreement with the earlier observations in perchloric acid medium[37,38]. The linear increase in absorbance at 460 nm with increase in PPH concentration showed that the protonated drug moiety, PPH+ had no effect on the complex formation and its colour stability.

Since the method is essentially the measurement of iron (III) thiocyanate complex, different variables like the kind of acid, source of iron (III), excess of thiocyanate and contact time which influence the colour intensity and the stability were optimised.

The effect of varying concentrations of HNO_3, $HClO_4$ and H_2SO_4 on the absorbance was studied. HNO_3 and $HClO_4$ gave similar sensitivities while the use of H_2SO_4 led to a much lower sensitivity. Perchloric acid was chosen as the reaction medium in preference to nitric acid for lower blank absorbance, and because low and erratic results were obtained with nitric acid. The effective perchloric acid concentration employed was about 0.5 M.

Different sources of iron (III) may be used, such as iron (III) nitrate[38], iron (III) ammonium sulphate[40] and iron (III) perchlorate[37]. Iron (III) nitrate was preferred to the other iron (III) salts because the procedure using iron (III) nitrate was found to be more sensitive and more linear than that using iron (III) ammonium sulphate and because of high chloride content of iron (III) perchlorate. One millilitre of iron (III) nitrate solution in a total volume of 10 ml was found adequate. Higher concentrations were found to increase the absorbance only slightly but larger blanks were obtained. With increasing concentrations of mercury (II) thiocyanate, increase in absorbance was only marginal. Two millilitre of reagent solution in a total volume of 10 ml was found sufficient.

Different solvents such as ethanol[38], methanol[41] and water[37] have been used to prepare mercury (II) thiocyanate reagent. High sensitivity was obtained when methanolic solution was used. Moreover, large amounts of ethanol are reported to bleach the iron (III) thiocyanate complex[37]. The reaction is fast and colour development is considered complete in 5 min at room temperature (30±2°). The colour remained stable upto 6 h.

Titrimetry was found applicable in the range 1-9 mg, outside which the results were not satisfactory. The relationship between the titration end-point obtained by the proposed titrimetric method and the drug amount was examined. The linearity between the amount of drug and titration end-point is apparent from the calculated correlation co-efficient, r (0.9986) obtained by the best fit line via linear least squares treatment. The calculated value of r shows that the reaction between $AgNO_3$ and PPH proceeds stoichiometrically in the ratio 1:1.

Under the described experimental conditions, a linear

correlation was obtained between absorbance (A) and the concentration (C) of PPH over the range 10-50 μg/ml. The linear regression equation was:

$$A = 0.0526+0.0061\ C \qquad (r = 0.9713)$$

The apparent molar absorptivity and Sandell sensitivity were $2.63x10^3$ l/mol.cm and 112.48 $ngcm^{-2}$, respectively. The limit of detection was 1.47 μg/ml and the limit of quantification as the lowest standard concentration which could be determined with acceptable accuracy and precision was 4.91 μg/ml.

The accuracy of the methods was evaluated by analysing the pure drug in different levels and the precision was established by determining the relative standard deviation of seven replicate analysis on the same solution containing three different levels of the drug. The percentage recovery, the RSD and the range of error (%) at 95% confidence level indicate the high accuracy and precision of the methods, Table 1.

To ascertain the ruggedness of the methods, four replicate determinations at different concentration levels of the drug were carried out. The within day RSD values were within 2%. The values of between-day RSD for different concentrations of drug, obtained from determinations carried out over a period of four days are given in Table 2 and indicate the reasonable precision of the methods.

Bromide ion interferes in any quantity. Higher concentration of sulphates and phosphates bleach the colour of iron (III) thiocyanate complex. Large amounts of ethyl and isopropyl alcohols are reported[37] to impart a yellowish brown colour to the complex. But none of the above substances is present in either the reagents employed or the formulations analysed, and hence the methods are devoid of error due to them.

The proposed methods were applied to the determination of PPH in tablets. The results presented in Table 3 indicate that excipients present in formulations do not interfere with the visual end-point detection and spectrophotometric measurement of iron (III) thiocyanate complex. The same batch of tablets were also assayed by BP method simultaneously and statistical analysis (t and F - tests) of the results obtained by the proposed methods and the reference method showed no significant difference in the performance of the two methods.

The procedures presented in this communication offer, in addition to satisfactory accuracy, a convenient solution to

TABLE 1: ACCURACY AND PRECISION OF THE METHODS.

Titrimetric Method					Spectrophotometric Method				
Amount taken, mg	Amount found* mg	Error, %	RSD, %	Range of error, %	Amount taken, μg	Amount found* μg	Error, %	RSD, %	Range of error, %
2	2.05	2.5	3.59	3.45	100	98.96	2.99	3.02	2.91
5	4.94	1.2	1.49	1.43	300	301.18	1.63	0.54	0.52
8	8.05	0.63	1.94	1.86	500	508.49	3.50	0.68	0.65

* Values obtained for seven determinations.

TABLE 2: BETWEEN-DAY PRECISION OF THE METHODS.

Titrimetry			Spectrophotometry		
Amount taken, mg	Amount found*, mg	RSD, %	Amount taken, μg	Amount found*, μg	RSD, %
2.0	2.07	1.32	100	100.52	1.45
6.0	6.01	0.55	200	199.63	2.91
9.0	8.96	0.18	400	406.43	1.06

* Values obtained for four determinations over a period of 4 days.

TABLE 3: RESULTS OF DETERMINATION OF PPH IN TABLETS.

Different Tablet brands	Label claim, mg	Found*, % recovery ± SD t-value (2077) F-Value (6.39)*		Reference method
		Titrimetry	Spectrophotometry	
B. P. Norm	40	97.9±1.61 t=1.11 F=6.32	98.2±0.82 t=1.20 F=1.64	98.7±0.64
Betablock	10	101.0±0.85 t=1.78 F=1.87	98.8±0.28 t=2.76 F=4.90	99.6±0.62
Betacap	10	98.8±1.21 t=1.08 F=6.35	100.5±0.85 t=2.45 F=3.13	99.4±0.48
Ciplar	40	102.2±0.76 t=2.19 F=1.79	101.0±0.36 t=0.98 F=2.59	101.3±0.58
Corbeta	40	98.7±0.96 t=2.77 F=2.39	99.7±0.86 t=0.58 F=1.92	100.1±0.62
Inderal	10	100.7±1.02 t=2.55 F=1.89	98.9±0.75 t=0.76 F=1.02	99.3±0.74
Propal	10	101.4±0.42 t=0.42 F=1.72	100.8±0.80 t=1.34 F=6.25	100.3±0.32

* Average of five determinations. * Figures in the parentheses are the tabulated values at 95% confidence level.

a number of problems associated with the determination of PPH. The notable advantages of the methods include long and dynamic concentration range, high stability of iron (III) thiocyanate colour for spectrophotometric measurement and reasonable within day and between-day precision. The procedures are not critically dependent on any experimental variable and the analysis can be completed in less than 20 mm and hence, suitable for routine analysis.

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REFERENCES

1. Klaus, F., Eds., In; Analytical Profiles of Drug Substances, Vol. 12, Academic Press Inc., New York, 1983, 325.
2. Hold, K.M., de Beer, D., Soeidirman, J.R., Zuidema, J. and Maes, R.A.A., **J. Pharm. Biomed. Anal.,** 1995, 13, 1401.
3. Zoest, A.R., Hung, C.T. and Wanwimolruk, S., **J. Liq. Chromatogr.,** 1992, 15, 1277.
4. Choi, Y.J., Seo, M.K., Kim, I.C. and Lee, Y.H., **J. Chromatogr. Biomed. Appln.,** 1997, 694, 415.
5. Egginer, G., Lindner, W., Vandembosch , C. and Lue Mursart, D., **Biomed. Chromatogr.,** 1993, 7, 277.
6. The British Pharmacopoeia, Her Majesty's Stationery Office, London, 1988, 996.
7. The U S Pharmacopoeia, Pharmaceutical Convention Inc., Rockville, MD, 1985, 907.
8. Sanghavi, N.M. and Vyas, J.J., **Indian Drugs,** 1992, 29, 7.
9. Raman, K. and Agrawal, Y.K., **Indian J. Pharm. Sci.,** 1989, 51, 144.
10. Sultan, S.M., **Analyst,** 1988, 113, 149.
11. Shingbal, D.M. and Prabhudesai, J.S., **Indian Drugs,** 1984, 21, 304.
12. Bedair, M.M., Galal, S.M. and El - Yazbi, F.A., **Sci. Pharm.,** 1992, 60, 235.
13. Shingbal, D.M. and Khandeparkar, A.S., **Indian Drugs,** 1987, 24, 365.

14. Husain, S., Krishnamurthy, A.S.R., Sekar, R. and Prasad, P.R., **Indian Drugs,** 1995, 32, 574.
15. Sastry, C.S.P., Srinivas, K.R. and Krishna Prasad, K.M., **Mikrochim. Acta,** 1996, 122, 77.
16. Radulovic, D., Jovanovic, M.S. and Zivanovic, L., **Pharmazie,** 1986, 41, 434.
17. Sastry, C.S.P., Sailaja, A., Rao, T.T. and Krishna, D.M., **Indian Drugs,** 1992, 29, 473.
18. Leml, J. and Knockeret, I., **Pharm. Weekbl. Sci.,** 1983, 142.
19. Batch, R.F. and Winefordner, J.D., **J. Pharm. Sci.,** 1983, 72, 559.
20. Sane, R.T., Gangrade, M.G., Bapat, V.V., Surve, S.R. and Chonkar, N.L., **Indian Drugs,** 1993, 30, 205.
21. Olsen, C.S., **Pharmacopoeial Forum,** 1987, 13, 2177.
22. Dasgupta, V. and Dhruv, A.B., **Drug Develop. Ind. Pharm.,** 1986,12, 691.
23. Gui, Z. and Wang, L., **Yaowu Fenxi Zazhi,** 1991, 11, 41.
24. Issa, M. and Amin, A.S., **Mikrochim. Acta,** 1995, 118, 85.
25. Vogel, A.I., In; A Text Book of Quantitative Inorganic Analysis, 3rd Edn., ELBS, London, 1969, 259.
26. Vogel, A.I., In; A Text Book of Quantitative Inorganic Analysis, 3rd Edn., ELBS, London, 1969, 265.
27. Andrey, M., and Mirimnoff, A., **Pharm. Acta Helv.,** 1960, 35, 276.
28. Zlakowrka, W., **Acta Pol. Pharm.,** 1982, 40, 55.
29. Alary, J., Bourbon. P., Escrient, C. and Vandaele, **J. Fresenius' Z. Anal. Chem.,** 1985, 322, 777.
30. John, P.F., **Talanta,** 1969, 16, 617.
31. Subert, J. and Havhickova., **Farm Obz.,** 1988, 57, 345.
32. Qiu, Z. and Chen, Z., **Yaowu Fenxi Zazhi,** 1984, 4, 159.
33. Vogel, A.I., In; A Text Book of Quantitative Inorganic Analysis, 3rd Edn., ELBS, London, 1969, 78.
34. Wilkinson, C.H. and Cole, R., **J. Hosp. Pharm.,** 1969, 26, 45.
35. Jin, H. and Yao, T., **Fenxi Hauaxue,** 1982, 10, 189.
36. Caldwell, J.R. and Moyer, H.V., **Ind. Eng. Chem, Anal.,** 1935, Ed. 7, 38.
37. Zall, D.M., Fisher, D. and Gamer, M.Q., **Anal. Chem.,** 1956, 28, 1665.
38. Florence, T.M. and Farrar, Y.J., **Anal. Chim. Acta,** 1971, 54, 373.
39. Krishnamurthy, R. and Bhagawat, K.A., **Indian J. Expt. Biol.,** 1990, 28, 198.
40. Lau, O.W. and Mok, C.S., **J. Sci. Food Agri.,** 1980, 31, 1275.
41. Herrero, M.A., Atiemza, J. and Maquieira, R.P., **Analyst,** 1992, 117, 1019.