Novel Spectrophotometric Estimation of Some Poorly Water Soluble Drugs Using Hydrotropic Solubilizing Agents

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Ultraviolet absorption spectrophotometric method for the estimation of poorly water soluble drugs like nalidixic acid, norfloxacin, tinidazole, and metronidazole in pharmaceutical formulations, has been developed. Aqueous solubilities of these selected model drugs were enhanced to a great extent (5 to 98 fold) in 2.0 M sodium benzoate, and in 2.0 M niacinamide solutions. The primary objective of the present investigation was to employ these hydrotropic solutions to extract the drugs from their dosage forms, precluding the use of costlier organic solvents. The selected λmax for nalidixic acid, norfloxacin, tinidazole, and metronidazole, were 330 nm, 324 nm, 318 nm and 320 nm, respectively. Sodium benzoate and niacinamide did not show any absorbance above 300 nm, and therefore, no interference in the estimation was seen. The results of analysis have been validated statistically, and by recovery studies. The proposed methods are new, simple, economic, accurate, safe, and precise.

Increasing the aqueous solubility of insoluble and slightly soluble drugs, is of major importance. Various techniques have been employed to enhance the aqueous solubility of poorly water-soluble drugs. Hydrotropic solubilization is one of them. The term hydrotropy has been used to designate the increase in solubility of various substances in water, due to the presence of large amounts of additives. sodium benzoate, niacinamide, sodium salicylate, sodium acetate, sodium citrate, and urea, have been employed to enhance the aqueous solubility of many poorly water soluble drugs[1-25]. Maheshwari analyzed various poorly water-soluble drugs, using hydrotropic solubilization phenomenon viz. cefixime,[1] frusemide[2], salicylic acid[3], ketoprofen[3-4], tinidazole[5], and aceclofenac[6]. Maheshwari *et al.* have developed various analytical techniques employing hydrotropic solubilization phenomenon, to analyze poorly water-soluble drugs, hydrochlorothiazide[7], ofloxacin[8], and aceclofenac[9].

Various organic solvents like methanol, chloroform, alcohol, dimethyl formamide, and benzene have been employed for the solubilization of poorly water soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity, pollution, and error, in analysis due to volatility. The primary objective of this study was to employ hydrotropic solubilizing agents, sodium benzoate and niacinamide for the selected model drugs to preclude the use of organic solvents. In the preliminary solubility studies, it was found that there was considerable enhancement in the aqueous solubilities of nalidixic acid, norfloxacin, tinidazole, and metronidazole, in 2.0 M sodium benzoate, and 2.0 M niacinamide solutions. Since sodium benzoate and niacinamide do not absorb above 300 nm, it was thought worthwhile to use these hydrotropic agents, to extract out the drugs having λmax above 300 nm, from their corresponding solid dosage forms. The spectrophotometric estimations of drugs were not affected in the presence of solublizing agents, sodium benzoate and niacinamide. Recovery studies and statistical analysis were used to validate the methods.

MATERIALS AND METHODS

A Shimadzu UV/Visible recording spectrophotometer (Model-UV160A) with 1 cm matched silica cells, were employed. Metronidazole, tinidazole, and norfloxacin, were obtained from M/s. Alkem Laboratories Limited, Mumbai, and nalidixic acid was procured from M/s.

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Ranbaxy Laboratories Limited, Dewas, as gift samples. All other chemicals were of analytical grade.

Preparation of standard solutions and calibration curves:

The standard solutions (200 μg/ml) of all the drugs were prepared in distilled water. In case of nalidixic acid, tinidazole, and norfloxacin, it was necessary to warm on a water bath to accelerate the dissolution process. The standard solutions (200 μg/ml) were diluted with distilled water, to obtain various dilutions (5, 10, 15, 20, 25, 30, 35 and 40 μg/ml). Solutions containing 15 μg/ml of drug were scanned between 200 nm and 400 nm. The λmax for metronidazole, tinidazole, norfloxacin, and nalidixic acid were found at 320 nm, 318 nm, 324 nm and 330 nm, respectively. A linear relationship was observed over the range of 5-30 μg/ml for metronidazole, 5-25 μg/ml for tinidazole, 5-35 μg/ml for norfloxacin, and 5-40 μg/ml for nalidixic acid.

Preliminary solubility studies of drugs:

Solubilities of all selected model drugs were determined at 28±1°. An excess amount of drug was added to screw capped 30 ml glass vials containing different aqueous systems viz. distilled water, buffer of pH 6.4, buffer of pH 8.2, 2.0 M sodium benzoate solution, and 2.0 M niacinamide solution. The vials were shaken mechanically for 12 h at 28±1°, in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hs, and then centrifuged for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatman filter paper No. 41. The filtrates were diluted suitably, and analyzed spectrophotometrically against corresponding solvent blank.

Analysis of the tablet formulations of the drugs by the proposed method:

Twenty tablets of metronidazole formulation–I were weighed, and ground to a fine powder. An accurately weighed powder sample equivalent to 50 mg of metronidazole, was transferred to a 25 ml volumetric flask. 20 ml of 2.0 M sodium benzoate solution was added, and the flask was shaken for about 10 min to dissolve the drug, and the volume was made up to the mark with distilled water. The solution was filtered through Whatman filter paper No. 41. The filtrate was divided into two parts A, and B. Part A was kept at room temperature for 48 h to check its chemical stability and precipitation, if any. Part B was diluted appropriately with distilled water, and was analyzed on a UV Spectrophotometer against reagent blank. The drug content of the tablet formulation was then calculated. There was no precipitation in Part A solution after 48 h. After 48 h (at room temperature), Part A solution was analyzed in the same way as Part B solution.

A similar procedure was used in case of tablet formulation II of metronidazole, tablet formulations III and IV of tinidazole, and tablet formulations V and VI of norfloxacin. Like 2.0 M sodium benzoate solution, 2.0 M niacinamide solution was also used to analyze all types of tablet formulations. In case of nalidixic acid tablet formulations VII and VIII, 40 ml of 2.0 M sodium benzoate, and 40 ml of 2.0 M niacinamide solutions were employed in 50 ml volumetric flasks, and their volumes were made up to the mark with distilled water. Table 1 shows the results of all such analyses.

Recovery studies:

For recovery studies, tablet powder (formulation I to VIII of drugs), equivalent to 50 mg drug was taken in a 25 ml volumetric flask. In this flask, 20 mg of pure drug (corresponding spiked drug) was transferred, 20 ml of 2.0 M sodium benzoate solution was added, and the flask was shaken for about 10 min. The volume was made up to the mark with distilled water, and filtered through Whatman filter paper No. 41. The solution was diluted appropriately with distilled water, and analyzed for drug content. In case of recovery studies for nalidixic acid formulations, 40 ml of 2.0 M sodium benzoate solution was used, and the volume was made up to 50 ml with distilled water. A similar procedure was repeated using 2.0 M niacinamide solution, in place of 2.0 M sodium benzoate solution, in all the cases. The results of analysis of recovery studies are presented in Table 2.

RESULTS AND DISCUSSION

Results of solubility studies indicated that, enhancements in aqueous solubilities in 2.0 M sodium benzoate solution, as compared to solubility in distilled water, were more than 5, 6, 40 and 98 fold in cases of metronidazole, tinidazole, norfloxacin, and nalidixic acid, respectively. Similarly, enhancement in aqueous solubility in 2.0 M niacinamide solution as compared to solubility in distilled water, were more than 10, 7, 5 and 21 fold in cases of metronidazole, tinidazole, norfloxacin, and nalidixic acid, respectively.

The pH of 2.0 M niacinamide solution was 6.4, and pH of 2.0 M sodium benzoate solution was 8.2. Therefore, in order to study the influence of pH on solubilities, buffer

TABLE 1: RESULTS OF ANALYSIS OF COMMERCIAL TABLET FORMULATIONS

Drug	TF	LC (mg/tab)	Using 2.0 M sodium benzoate solution			Using 2.0 M niacinamide solution		
			% LC estimated* (mean±S.D.)	% Coeff. of variation	SE	% LC estimated* (mean±S.D.)	% Coeff. of variation	SE
A	I	400	101.0±0.36	0.36	0.21	101.2±0.89	0.88	0.52
	II	400	99.9±0.69	0.69	0.40	98.9±0.91	0.92	0.53
B	III	400	100.9±0.83	0.83	0.48	99.9±0.76	0.76	0.44
	IV	400	100.3±0.93	0.93	0.54	100.9±0.90	0.90	0.52
C	V	300	101.8±1.03	1.02	0.60	101.4±0.89	0.88	0.51
	VI	300	100.0±1.81	1.81	1.05	98.9±1.10	1.11	0.64
D	VII	500	101.7±0.80	0.79	1.02	100.4±0.24	0.24	0.14
	VIII	500	100.8±0.68	0.68	1.01	98.4±0.67	0.68	0.39

A- Metronidazole, B- Norfloxacin, C- Tinidazole and D- Nalidixic acid, TF- Tablet formulation, LC- Label claim, SE- Standard error, *Mean of three determinations, I- Flagyl-400 tablets (Nicholas Piramal India Limited), II- Metrogyl-400 tablets (Unique Pharmaceutical Laboratories, India), III- Norflox-400 tablets (Cipla Limited, India), IV- Uroflox-400 tablets (Torrent Pharmaceuticals Limited, India), V- Tini-300 tablets (Kopran Limited, India), VI- Tiniba-300 tablets (Cadila Health Care, India), VII & VIII- Two different batches of Gramoneg tablets (Ranbaxy Laboratories Limited, India).

TABLE 2: RECOVERY STUDY FOR SPIKED CONCENTRATION OF DRUGS ADDED TO THE PREANALYZED DOSAGE FORM

Drug	TF	AD (mg)	Drug added (spiked) (mg)	Using 2.0 M Sodium benzoate solution			Using 2.0M niacinamide solution		
				% Recovery estimated* (mean±S.D.)	% Coeff. of variation	SE	% Recovery estimated* (mean±S.D.)	% Coeff. of variation	SE
A	I	50	10	99.6±0.71	0.72	0.41	100.3±0.99	0.99	0.57
	II	50	10	99.3±1.04	1.05	0.60	99.7±1.38	1.38	0.80
B	III	50	10	99.1±0.81	0.82	0.47	100.3±0.38	0.38	0.22
	IV	50	10	100.4±0.58	0.58	0.34	99.3±0.77	0.77	0.44
C	V	50	10	98.1±0.68	0.70	0.40	100.9±0.59	0.59	0.34
	VI	50	10	99.7±1.04	1.05	0.60	99.2±1.32	1.33	0.76
D	VII	50	10	100.9±0.94	0.93	0.56	99.7±0.80	0.80	0.46
	VIII	50	10	99.1±0.63	0.64	0.36	101.2±0.89	0.87	0.51

A- Metronidazole, B- Norfloxacin, C- Tinidazole and D- Nalidixic acid, TF- Tablet formulation, AD- Amount of drug, LC- Label claim, SE- Standard error, *Mean of three determinations, I- Flagyl-400 tablets (Nicholas Piramal India Limited), II- Metrogyl-400 tablets (Unique Pharmaceutical Laboratories, India), III- Norflox-400 tablets (Cipla Limited, India), IV- Uroflox-400 tablets (Torrent Pharmaceuticals Limited, India), V- Tini-300 tablets (Kopran Limited, India), VI- Tiniba-300 tablets (Cadila Health Care, India), VII & VIII- Two different batches of Gramoneg tablets (Ranbaxy Laboratories Limited, India).

solutions of pH 6.4 and 8.2 were made, and the solubilities of all the drugs were determined. In case of metronidazole, tinidazole, and norfloxacin, there was negligible effect on solubilities in buffer solutions, as compared to their solubilities in water. This study proves that increase in solubilities of these three drugs in hydrotropic solutions (2.0 M sodium benzoate and 2.0 M niacinamide), are not due to alteration in pH, but are due to hydrotropic phenomenon. The solubilities of nalidixic acid in distilled water and buffer of pH 6.4, were nearly same. However, the solubility of nalidixic acid in buffer of pH 8.2 was increased more than 10 fold, but its solubility in 2.0 M sodium benzoate solution (pH 8.2), was more than 98 fold. This indicates, that the enhancement in the aqueous solubility of nalidixic acid in 2.0 M sodium benzoate solution, was largely due to hydrotropy.

Part A solutions of all drugs were kept at room temperature for 48 h. There was no precipitation of drugs in Part A solutions within 48 h. In addition, drug contents of Part A solutions (after 48 h) were same as those of Part B solutions (fresh solutions). This study reveals that the estimations can be done within 48 h at least, without having any detrimental effect on drug stability.

From Table 1, it is evident that there is good agreement between the amounts estimated, and those claimed by the manufacturers. Percent label claims are very close to 100, with low values of standard deviation, % coefficient of variation, and standard error.

Accuracy, reproducibility, and precision of the proposed methods, were further confirmed by percent recovery values, which were close to 100 with low values of standard deviation, % coefficient of variation, and standard error (Table 2).

From this study, it is obvious that there was no interference of sodium benzoate and niacinamide in the estimation of norfloxacin (λmax–324 nm), nalidixic acid (λmax–330 nm), metronidazole (λmax–320 nm), and tinidazole (λmax–318 nm). Sodium benzoate and

niacinamide do not absorb above 300 nm. Because of these reasons, it can be concluded, that a large number of poorly water soluble drugs having λmax above 300 nm, may be tried for estimation by the proposed method, provided that their preliminary solubility studies are conducted to observe the enhancement effect on solubility. Sodium benzoate solution is cheaper than most of the organic solvents, and can thus substitute expensive methanol, dimethyl formamide, chloroform and carbon tetrachloride. Drawbacks of organic solvents include toxicity, error due to volatility, pollution, and cost. Thus 2.0 M solutions of sodium benzoate and niacinamide may be better substitutes for organic solvents.

It is thus concluded, that the proposed method is new, simple, cost effective, accurate, safe, free from pollution and precise, and can be successfully employed in the routine analysis of these drugs in pharmaceutical dosage forms. The proposed method shall prove equally effective to analyze metronidazole, nalidixic acid, norfloxacin and tinidazole, in the corresponding drug samples (basic drugs), and may prove to be of great importance in pharmaceutical analysis.

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REFERENCES

1. Maheshwari, R.K., **The Indian Pharmacist**, 2005, Vol. IV (No. 36), 63.
2. Maheshwari, R.K. **The Indian Pharmacist**, 2005, Vol. IV (No. 34), 55.
3. Maheshwari, R.K., **Asian J. Chem.**, (In Press).
4. Maheshwari, R.K., **The Pharma Review**, 2005, 3, 123.
5. Maheshwari, R.K. **Asian J. Chem.**, (In Press).
6. Maheshwari, R.K., **Asian J. Chem.**, (In Press).
7. Maheshwari, R.K., Chaturvedi, S.C. and Jain, N.K., **Indian Drugs**, 2005, 42, 541.
8. Maheshwari, R.K., Chaturvedi, S.C. and Jain, N.K., **Indian Drugs**, 2005, 42, 760.
9. Maheshwari, R.K., Chaturvedi, S.C. and Jain, N.K., **Indian Drugs**, (In Press).
10. Jain, N.K. and Patel, V.V., **Eastern Pharmacist**, 1986, 29, 51.
11. Jain, N.K., Agrawal, R.K. and Singhai, A.K., **Pharmazie**, 1990, 45, 221.
12. Higuchi, T. and Drublis, A., **J. Pharm. Sci**., 1961, 50, 905.
13. Agrawal, S., Pancholi, S.S., Jain, N.K. and Agrawal, G.P., **Int. J. Pharm.**, 2004, 274, 149.
14. Ueda, S. **Chem. Pharm. Bull.**, 1966, 14, 2.
15. Poochikian, G.D. and Cradock, J.C., **J. Pharm. Sci.,** 1979, 68, 728.
16. Miyahara, M. and Takahasi, T., **Chem. Pharm. Bull.**, 1982, 30, 288.
17. Drawish, A., Florence, A.T. and Saleh, A.M., **J. Pharm. Sci.,** 1989, 78, 577.
18. Etman, M.A. and Hada, A.H., **Acta Pharm.**, 1999, 49, 291.
19. Saleh, A.M. and Daabis, N.A., **Pharmazie**, 1974, 29, 525.
20. Hussain, M.A., Diluccio, R.L. and Maurin, M.B., **J. Pharm. Sci.,** 1993, 82, 77.
21. Ibrahim, S.A., Ammar, H.O., Kasem, A.A. and Abu-zaid, S.S., **Pharmazie**, 1979, 34, 809.
22. Frost, D.V., **J. Am. Chem. Soc**., 1947, 69, 1064.
23. Badwan, A.A., El-Khordagui, L.K. and Khalil, S.A., **Int. J. Pharm**., 1983, 13, 67.
24. Elsamaligy, M.S., Hazma, Y.E. and Abd-Elgawad, N.A., **Pharm. Ind.,** 1994, 54, 474.
25. Rasool, A.A., Hussain, A.A. and Dittert, L.W., **J. Pharm. Sci.,** 1991, 80, 387.

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