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Hydrotropic solubilization phenomenon spectrophotometric estimation of Tenfovir disoproxil fumerate tablet

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Abstract

In the present investigation, 2.0 M sodium benzoate solutions was employed as hydrotropic solubilizing agent to solubilize poorly water-soluble drug Tenfovir disoproxil fumerate for its spectrophotometric analysis. 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.Ultraviolet absorption spectrophotometric method for the estimation of poorly water soluble drugs like Tenfovir disoproxil fumerate in pharmaceutical formulations has been developed. Aqueous solubilities of this selected model drugs was to a great extent (112 to 121 fold) in 2.0 M sodium benzoate solutions. The selected max for Tenfovir disoproxil fumerate were 317 nm respectively. The hydrotropic solutions used did not show any absorbance above 332 nm, and therefore, no interference in the estimation was seen. The results of analysis have been validated statistically, and by recovery studies.

Keywords: Hydrotropic Solubilization, Tenfovir disoproxil fumerate, sodium benzoate,

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Introduction

Tenfovir disoproxil fumerate is a novel drug used in combining fixed doses of the nucleoside reverse transcriptase inhibitor emtricitabine and with the non-nucleoside reverse transcriptase inhibitor efavirenz represents the first once-daily, one-tablet antiretroviral regimen. [1-4] Tenfovir disoproxil fumerate is chemically know as 9-[(R)-2-[[bis[[isopropoxy carbonyl] oxy] methoxy]phosphonyl]methoxy]popyl]adenine fumarate. Various techniques have been employed to enhance the aqueous solubility of poorly water soluble drugs such as alteration in pH of solvent, co-solvents, complexation, hydrotropic solubilization etc. The term "hydrotrophy"

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originally put forwarded by Newberg[5] to describe the increase in the solubility of the solute by the addition of fairly high concentration of alkali metal salts of various organic acids. According to Newberg, the solublizing agent, are anionic organic salts. Saleh and El-Khordagui[6] made an attempt to extent the definition of the term hydrotropic agent to included cationic and nonionic organic compounds bearing the essential structural features of Newberg's hydrotropes. Cationic compounds such as p-amino benzoic acid hydrochloride, procaine hydrochloride and neutral molecules such as resorcinol and pyrogallol confer typical hydrotropic properties.Winsor[7] considered hydrotrophy as a solubilization phenomenon, with the solute dissolved in oriented clusters are the hydrotropic agents. Sodium salicylate, sodium benzoate, sodium lauryl sulphate, sodium glycinate, sodium gentisate, nicotinamide, urea, sodium acetate, sodium citrate and niacinamide have been employed as a hydrotropic agent[8-13]. A successful attempt have been made by using this phenomenon for the analysis of various poorly water soluble drugs viz. frusemide, cefixime, hydrochlorothiazide, ketoprofen, bulk sample of ketoprofen and salicylic acid, norfoxacin in combination with tinidazole and metronidazole[14-20].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. None of these methods are without their limitations so the need was felt to develop three new, simple, accurate, environmental friendly, cost effective, safe, sensitive spectrophotometric methods for simultaneous estimation of Tenfovir disoproxil fumerate in tablet dosage form by using aqueous solution of 2.0 M sodium benzoate solution, as a hydrotropic agent.

Materials and Methods

UV-visible double beam spectrophotometer, Shimadzu model 1700 with spectral bandwidth of 1 nm, wavelength accuracy of ± 0.3 nm and a pair of 10 mm matched quartz cells was used. Tenfovir disoproxil fumerate was obtained as a gift sample from Hetero Drugs Ltd, Hyderabad. The commercially available tablets, (Tencef, Hetero) were procured from local market. All chemicals and solvents used were of analytical grade.

Preliminary solubility studies of drugs
Solubility of drug was determined at 25 ±1°C. An excess amount of drug was added to three screw capped 30 ml glass vials containing different aqueous system viz. distilled water, buffer of pH 9.1, 2.0M Sodium benzoate solution. The vials were shaken for 10 hrs at 25±1°C in a mechanical shaker. These solutions were allowed to equilibrate for the next 24 hrs and then centrifuged for 15 minutes at 1500rpm. 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.

Preparation of standard stock, calibration curve
Accurately weighed 50mg of Tenfovir disoproxil fumerate was solubilized by 50 ml of 2.0M sodium benzoate solutions and final volume was adjusted with distilled water in 100 ml of volumetric flask. From the above solution 10ml of solution was taken and diluted to 50 ml with distilled water to get a solution containing 100 µg/ml of each drug

It was necessary to warm on a water bath to accelerate the dissolution process. Working standard solutions were scanned in the entire UV range of 400-200 nm to determine the λ max of drugs. The λ max of Tenfovir disoproxil fumerate were found to be 317 nm respectively.The standard solutions were diluted with distilled water to obtain various dilutions (5, 10, 15, 20, 25, 30, 35µg/ml). The λmax of Tenfovir disoproxil fumerate was found at 317 nm. The linear

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relationship was observed over the range of 5-35 µg/ml. of drug. Absorbances were noted at 323 nm against corresponding reagent.

Analysis of the tablet formulations of the drug by proposed method:
Twenty tablets of Tenfovir disoproxil fumerate formulation were weighed, and ground to a fine powder. An accurately weighed powder sample equivalent to 300 mg of Tenfovir disoproxil fumerate was transferred to a 100 ml volumetric flask. 100 ml of 2.0 M sodium benzoate solution was added, and the flask was shaken for about 25 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 39 hrs 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 39 hrs. After 39 hrs (at room temperature), Part A solution was analyzed in the same way as Part B solution.

Recovery studies
In order to check the accuracy, reproducibility and the precision of the proposed method, recovery studies were conducted. Pre analyzed tablet powder (formulation-I) equivalent to 50 mg of Tenfovir disoproxil fumerate was transferred to a 100 ml volumetric flask. Pure Tenfovir disoproxil fumerate drug sample (20 mg) was added to the same volumetric flask. Now 40 ml of 2.0 M sodium benzoate solution was added and the flask was shaken for about 5 min to solubilize the drug. Then volume was made upto the mark with distilled water. Then solution was filtered through Whatman filter paper # 41. The filtrate was diluted with distilled water appropriately and absorbance was noted at 298 nm against corresponding reagent blank. Drug content was calculated and % recovery was calculated (Table II). Similar procedure was repeated using 30 mg and 40 mg of pure Tenfovir disoproxil fumerate as spiked concentration (in place of 20 mg). Recovery studies using formulation II and III in the same way. The drug contents were determined and % recoveries were estimated (Table II).

Table I: Results of analysis of commercial tablet formulations

Hydrotropic Solution	T F	LC (mg/tab)	estimated* (Mean±S.D)	Coeff. of variation	S.E.
2.0 M sodium benzoate	I	300	99.99±0.08	0.33	0.32
2.0 M sodium benzoate	II	300	100.3±0.22	0.65	0.12
2.0 M sodium benzoate	III	300	101.1±0.01	0.97	0.71

*TF- Tablet formulation, LC- Label claim, SE- Standard error, *Mean of three determinations*

Table II: Recovery study for spiked concentration of drugs added to the pre analyzed dosage form

Hydrotropic Solution	T. F	A.D	Drug Added (spiked mg)	estimated* (Mean±S.D)	Coeff. of variation	S.E.
2.0Msodium benzoate	I	300	20	100± 0.2	0.12	0.76
2.0 M sodium benzoate	II	300	30	99.98±0.14	0.36	0.41
2.0 M sodium benzoate	III	300	40	100±0.65	0.21	0.19

T.F- Tablet formulation, A.D- Amount of drug, S.D Standard deviation, S.E- Standard error

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Results and Discussion

Results of solubility studies indicated that enhancement in aqueous solubilities of Tenfovir disoproxil fumerate in 2.0 M sodium benzoate solution were more than 112 and 121 folds, respectively as compared to their solubilities in distilled water. Therefore, this solution was employed to extract Tenfovir disoproxil fumerate from the fine powder of tablet formulation. The pH of hydrotropic solutions was ranges from 8.0 to 9.2. Therefore, in order to study the influence of pH on solubilities, buffer solutions of pH 8.0 to 9.2 were made, and the solubilities of all the drugs were determined. This study proves that increase in solubilities of hydrotropic solutions are not due to alteration in pH, but are due to hydrotropic phenomenon. This indicates that the enhancement in the aqueous solubility of Tenfovir disoproxil fumerate in 2.0 M sodium benzoate hydrotropic solutions was largely due to hydrotropy. Part A solution of drug was kept at room temperature for 39 hrs. There was no precipitation of drug in Part A solutions within 39 hrs. In addition, drug contents of Part A solutions (after 39 hrs) were same as those of Part B solutions (fresh solutions). This study reveals that the estimations can be done within 39 hrs at least, without having any detrimental effect on drug stability. From Table I, 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 100.03, 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 I). From this study, it is obvious that there was no interference of hydrotropic solutions in the estimation of Tenfovir disoproxil fumerate (λmax 317 nm) hydrotropic solutions do not absorb above 332 nm. Because of these reasons, it can be concluded, that a large number of poorly water soluble drugs having λmax above 332 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. Hydrotropic solutions are cheaper than most of the organic solvents and can thus substitute expensive methanol, dimethyl formamide, chloroform and carbon tetrachloride.

Acknowledgment

References

[1] Budhavari S., The Merck Index., **2006**,14,1573.
[2] Sean Sweetman C.,Martindale-The Complete Drug Reference, **2005**, 34: 632.
[3] C.Sean Sweetman., Martindale The Complete Drug Reference, **2005**, 34:654.
[4] Indian pharmacopoeia. **2007**, 2: 1782.
[5] Neuberg C., *Biochem. Z.,* **1916**, 76: 107-176.
[6] Saleh A.M and Khordagui L.K., *Int. J. Pharm.,* **1985**, 24: 231-238.
[7] Winsor P.A., *Trans Faraday Soc*., **1950**, 54:762-772.
[8] Badwan A.A., El-Khordagui L.K., Saleh A.M. and Khalil S. A., *Int. J. Pharm.*, **1983**, 13: 67-74.
[9] Saleh A. M., Badwan A. A. and El-Khordagui L. K., *Int. J. Pharm*.,**1983**,17: 115-119.
[10] Hamaza Y. E. and Paruta A.N., *Drug Dev. & Ind. Pharm*., **1985**, 11: 1577-1596.
[11] Renee E.Coffmon and Done O. K., *J. Pharm. Sci*., **1996**, 13: 951-954.
[12] Maheshwari R. K*., Asian J.Chem*., **2006**, 18 (1);640-644.

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[13] Maheshwari R.K., Chaturvedi S.C. and Jain N. K., *Indian J. Pharm. Sci.,* **2006**, 68(2): 195-198.

[14] Maheshwari R. K., *The Indian Pharmacist*., **2005**, 34 (IV): 55-58.

[15] Maheshwari R. K., *The Indian Pharmacist*., **2005**, 36 (IV): 63-68.

[16] Maheshwari R. K., Chaturvedi S.C. and Jain N. K., *Indian Drugs*., **2005**, 42(8):541-544.

[17] Maheshwari R K, *The Pharma Review*., **2005**, 10:123-125.

[18] Maheshwari R. K., *Asian J. Chem*., **2006**, 18(1), 393-396.

[19] Maheshwari R.K., Maheshwari R.B and P. Bhatt, *Asian J. Chem*., **2006**, 18(2):1481-1486.

[20] Maheshwari R. K., Chaturvedi S.C.and Jain N. K., *Indian J. Pharm. Sci.*, **2006**, 68(2):195-198.