Bulletin of Pharmaceutical Research 2011;1(2):15-21
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

RESEARCH ARTICLE

APPLICATION OF MIXED HYDROTROPY IN SPECTROPHOTOMETRIC ANALYSIS OF FRUSEMIDE IN DIFFERENT FORMULATIONS

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Received: August 08, 2011 / Revised: August 11, 2011 / Accepted: August 12, 2011

The present study describes the use of an aqueous solution containing a blend of hydrotropic solubilizing agents (mixed hydrotropic substance's solution) as a successful solvent system utilizing the concept of mixed hydrotropy for spectrophotometric analytical estimation of various conventional formulations as well as novel drug delivery systems. Frusemide, a poorly water-soluble drug, was estimated by application of mixed hydrotropic solubilization method. There was more than 15-fold enhancement in aqueous solubility of frusemide in a solution of blend of hydrotropic agents which consisted of 30% urea, 13.6% sodium acetate and 11.8% sodium citrate. This solvent mixture was employed to solubilize the drug from the fine powder of tablet formulations as well as the niosomes of frusemide. The selected λ_{max} for spectrophotometric estimation was 333 nm. The hydrotropic agents used in the analysis and additives used in the manufacture of tablets and preparation of niosomes did not interfere in the analysis. Statistical data proved the accuracy, reproducibility and precision of the proposed method. The results suggested that proposed method is new, rapid, simple, accurate, and reproducible as well as employed aqueous solvent instead of organic solvents in estimation of drug from the dosage forms.

Key words: Mixed hydrotropy, Frusemide, Urea, Hydrotropic agents, Niosomes, Sodium acetate.

INTRODUCTION

The term hydrotropy originally put forward to describe the increase in the solubility of a solute by the addition of fairly high concentration of alkali metal salts of organic acids (Neuberg, 1916). Later on, the definition of the hydrotropic agent was extended to include cationic and non-ionic organic compounds bearing the essential structural features of Newberg's hydrotropes (Saleh and El-Khordagui, 1985). The efficacy of hydrotropes in enhancing the water solubility of pharmaceutical compound depends on suitably matching the structural features of the hydrotropic agents with those of the drug. Each hydrotropic agent is effective in increasing the water solubility of selected hydrophobic drugs. The effective hydrotropic agents are those that destabilize water structure and at the same time interact with poorly soluble drug.

Maheshwari utilized application of hydrotropes in pharmaceutical analysis (Maheshwari *et al* 2005a; 2006a). Analysis of various poorly water-soluble drugs like ketoprofen, salicylic acid, aceclofenac and frusemide by titrimetric analysis by use of sodium benzoate solution as solubilizing agent is reported (Maheshwari, 2005; 2006a; 2006b). Also use of sodium benzoate as hydrotropic solubilizing agent to estimate various poorly water-soluble drugs such as ofloxacin, norfloxacin, nalidixic acid, metronidazole,

diclofenac sodium and tinidazole (Maheshwari *et al* 2005b; 2006b; 2011a) by spectrophotometric analysis and aceclofenac by titrimetric analysis was done (Maheshwari *et al* 2011b). The new application of hydrotropy is its employment in solubilizing the drug from the fine powder of tablet formulations for its quantitative analysis. Hydrotropic agents act nicely at higher concentrations (10% to 45%). Therefore, in mixed hydrotropy, mixture of two or more hydrotropic agents, in place of one, is utilized so that higher concentration of one hydrotropic agent can be replaced by smaller amounts of two or more hydrotropic agents; thus minimizing individual toxic concentration of single hydrotropic agent and at the same time, utilizing possible synergistic effect of mixed hydrotropes to obtain even greater solubility than one hydrotrope. Frusemide is a high ceiling loop diuretic and is a drug of choice for mobilizing edema fluid, in congestive heart failure and as an adjuvant in treatment of hypertension (Florey, 1989). Chemically, frusemide is 4-chloro-*N*-furfuryl-5-sulfamoylanthranilic acid, which is a derivative of 2-aminobenzoic acid with molecular formula $C_{12}H_{11}ClN_2O_5$ (**Figure 1**).

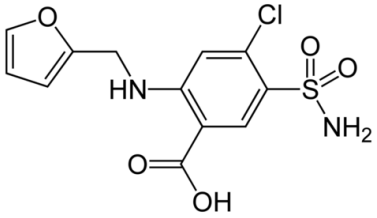

Fig. 1. Chemical structure of frusemide

There exists titrimetric method for the frusemide assay (Indian Pharmacopoeia, 2007), HPLC method for frusemide tablets assay (United States Pharmacopoeia, 2007) and UV spectrophotometric method (Indian Pharmacopoeia, 2007; British Pharmacopoeia, 2002) for frusemide assay. Estimation methods of drug content and encapsulation efficiency of targeted drug delivery systems like liposomes, niosomes, microspheres, nanoparticles use various toxic organic solvents. In the present study, mixed hydrotropic solution is used for estimation of drug content and % encapsulation efficiency from niosomes of frusemide.

MATERIALS AND METHODS

Chemicals

Frusemide was generously supplied by Alkem Laboratories Limited, Mumbai (India). Cholesterol (Sunchem, India), surfactant span 80 and diethyl ether (Oxford Laboratory, India) were used. Tablets of frusemide (Lasix 40 mg) were obtained commercially from local market. Urea, sodium citrate and sodium acetate were purchased from Qualigens, India Ltd. The dialysis membrane (cellulose membrane, 12400 mw cut off, Sigma chemicals) was used for dialysis. All the chemicals used were of analytical grade and double distilled water was used throughout the studies.

Instrumentation and analytical conditions

Present study was performed on UV Spectrophotometer (UV-1800, Shimadzu) at 333 nm and using 1.0 cm quartz cells. UV Probe Ver 2.31 software was used for all absorbance measurements.

Preliminary solubility studies of drug

Solubility of frusemide was determined at 25±1^{o}C. An excess amount of drug was added to screw capped 30 ml glass vials containing different aqueous systems such as distilled water, buffer (pH 4.0 and 8.2), and different individual hydrotrope solutions and in different blends of hydrotropic agents selected from individual hydrotropes at 25±1^{o}C.

Niosomes

Preparation of niosomes

Niosomes were prepared by ether injection method which involved slow injection of surfactant (span 80):cholesterol (150 *μ*mol) solution in ether (20 ml) through a 14 gauge needle at rate 0.25 ml/min in to a preheated 4.0 ml aqueous phase maintained at 60^{o}C. Ten mg frusemide was incorporated in ethereal solution of surfactant and cholesterol. The unentrapped (free) frusemide was removed by extensive dialysis. The prepared niosomes were placed in dialysis bag and free frusemide was dialyzed for 30 min each time in 100 ml of mixed hydrotropic solution (selected by solubility studies *i.e.* 30:13.6:11.8% *w/v* urea:sodium acetate:sodium citrate). The dialysis of free frusemide was almost completed after 12-15 changes when no frusemide was detectable in the recipient solution. The vesicle formation was confirmed by microscopic analysis and the ability of vesicles to entrap frusemide was examined by UV spectrophotometry (Vyas and Khar, 2008).

Average vesicular size

A film of vesicle dispersion fixed with 10% *w/v* gelatin solution, was observed under a light

microscope (Laborlux) at a magnification of 1000x and 1500x. About 100 vesicles were selected at random and their size was measured using previously calibrated ocular micrometer.

Preparation of the standard solutions

The standard stock solution of frusemide was prepared by solubilizing 100 mg of frusemide in 10 ml of blend of hydrotropic agents (30:13.6:11.8% *w/v* urea:sodium acetate:sodium citrate) and further diluted with distilled water up to 100 ml. The working standard solutions of concentrations 10 to 120 *μ*g/ml were prepared by diluting the stock solution with mixed hydrotropic solution.

Preparation and analysis of sample solutions

Analysis of frusemide as per Indian Pharmacopoeia (IP) method

Twenty tablets of frusemide were weighed and powdered. A quantity of the powder containing about 0.1 g of frusemide was weighed accurately and shaking was done with 150 ml of 0.1 M sodium hydroxide for 10 min. Then sufficient sodium hydroxide (0.1 M) was added to produce a volume of 250 ml and resulting solution was then filtered. From this, 5.0 ml was withdrawn and further diluted to 200 ml using sodium hydroxide (0.1 M). The absorbance of the resulting solution was measured at 271 nm.

Analysis of tablet formulation of frusemide by the proposed method

Ground powder of tablets of frusemide equivalent to 50 mg of frusemide was taken in a 25 ml volumetric flask, 20 ml blend of hydrotropic agents was added and shaking was done to solubilize the drug. Volume was made up to 25 ml with distilled water and filtered through Whatmann filter paper #41. The filtrate was divided into two parts. Part A was kept at room temperature for 24 h to check its chemical stability and precipitation, if any. Part B was diluted sufficiently with distilled water and was analyzed spectrophotometrically. Drug content was calculated followed by analysis of Part A solution after 24 h in the same way. Two different brands of tablets were analyzed separately.

Analysis of drug content in niosomes by proposed method

The percentage drug content and percentage entrapment efficiency was determined by dissolving 1 ml of the niosomes (obtained from dialysis) in 10 ml mixed hydrotropic solution and further diluting to 100 ml with same solution. Again 2 ml of this solution was further diluted to 10 ml with same mixed hydrotropic solution and absorbance of the clear solution was measured at 333 nm. Percentage drug content was expressed as the percentage of frusemide trapped in niosomes to the total amount of niosomes (obtained from dialysis). The percentage entrapment efficiency was expressed as the percentage of frusemide trapped in niosomes referred to the total amount of frusemide used. Similarly, the analysis was done using 0.1 M NaOH as a solvent instead of hydrotropic solution and analyzed at 271 nm.

Recovery studies

Recovery studies were performed by adding measured small amount of drug to pre-analyzed tablet powder equivalent to 100 mg drug and analyzed by the proposed method. The percentage recoveries for spiked amounts were calculated.

Method validation

The methods were validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. Analysis of variance (ANOVA) was used to verify the validity of the methods.

Linearity

The calibration curve was obtained with twelve concentrations of the standard solution (10-120 *μ*g/ml). The solutions were prepared in triplicate and the linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples at same concentration during the same day. The intermediate precision was studied by comparing the assays on different days (3 days). Six sample solutions were prepared and analyzed.

Accuracy

The accuracy was determined by recovery of known amounts of frusemide drug added to the samples at the beginning of the process. An accurately weighed amount of powder of tablets and niosomes equivalent to 20 mg of frusemide was transferred to 200 ml volumetric flask

separately and dissolved in mixed hydrotropic solution (30% urea, 13.6% sodium acetate, 11.8% sodium citrate) to make a final concentration of 100 μg/ml. Aliquots of 4.0 ml of this solution were transferred in to 20 ml volumetric flasks containing 2.0, 4.0 and 6.0 ml of frusemide standard solution (200 μg/ml) and mixed hydrotropic solution was added to make up the volume to give final concentrations of 40, 60 and 80 μg/ml. All solutions were prepared in triplicate and assayed. The percentage recovery of added frusemide drug was calculated using the proposed equation.

Limit of detection and limit of quantitation (LOD and LOQ)

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation.

RESULTS AND DISCUSSION

Method development

The UV absorption spectra of frusemide were monitored in different individual hydrotropic agents and solution of blend of hydrotropic agents. The solution consisting of 30:13.6:11.8% *w/v* urea:sodium acetate:sodium citrate at 333 nm in the measuring wavelength range of 200-400 nm with a fixed slit width of 5 nm was selected. UV spectrum of frusemide in blend of hydrotrope solution (30:13.6:11.8% *w/v* urea-sodium acetate-sodium citrate) is shown in **Figure 2**. No interfering absorbances were found due to the different hydrotropic agents in blend and excipients of frusemide tablet (**Figure 3**).

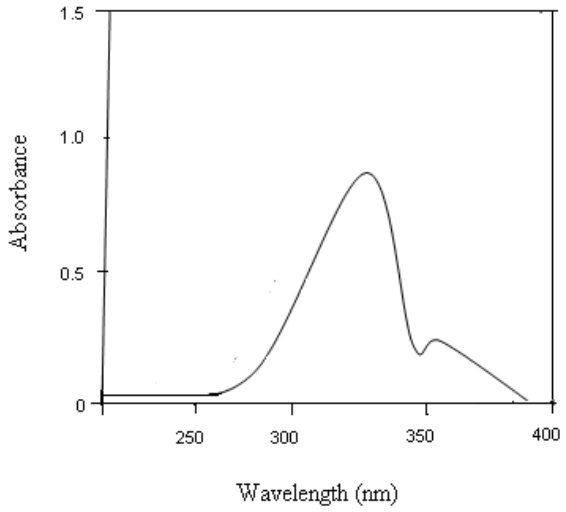

Fig. 2. UV spectrum of frusemide in blend of hydrotrope solution (30:13.6:11.8% *w/v* urea-sodium acetate-sodium citrate)

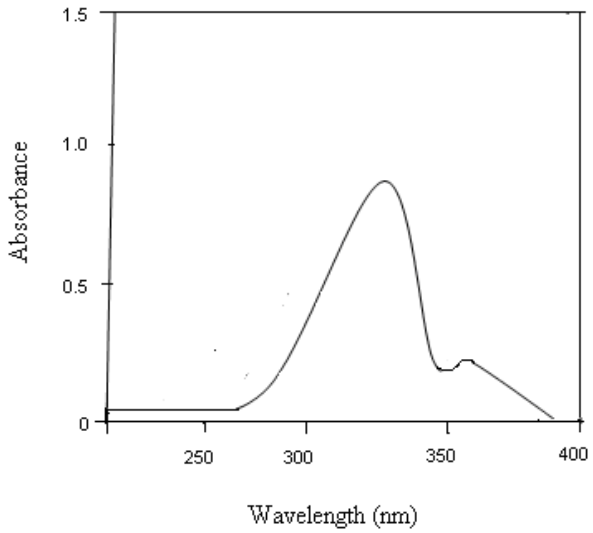

Fig. 3. UV spectrum of sample contained in commercial frusemide tablet in blend of hydrotrope solution (30:13.6:11.8% *w/v* urea-sodium acetate-sodium citrate)

Preparation of the standard solution and calibration curve

Five level calibration series with six analyses at each concentration level were measured for UV determination. The standard calibration curves of frusemide were constructed by plotting absorbance *vs* concentration for both individual hydrotropic agent's solution and solutions of blend of hydrotropic agents in six different days. The results were averaged and analyzed by linear simple regression model of $y = mx + c$ by the least-squares method. For all the calibration curves, a good linearity within the concentration range of 10-100 μg/ml for individual hydrotropic solutions and for mixed hydrotropic solution showed in range of 20-120 μg/ml. The mean regression equation of calibration curve and correlation coefficient obtained for mixed hydrotropic solution 30:13.6:11.8% *w/v* urea:sodium acetate:sodium citrate is shown in **Table 1**. The LOD and LOQ were found to be 19.48 and 59.05 ng/ml, respectively. The precision of the method, expressed as the relative standard deviation (RSD = 100 × SD/mean), was assessed. The RSD values were lower than 10%. Repeatability was given as intra-day precision and accuracy.

The frusemide contains a weakly absorbing chromophore in its molecule. Therefore, low frusemide concentrations were not detectable by UV (British Pharmacopoeia, 2002). Also, low concentrations showed poor linearity as it emerged the occurrence of scattering at these frusemide concentrations. Therefore, the

Table 1. Different parameters of the calibration curve of frusemide in selected solvent medium by UV-VIS spectrophotometry*

S. No.	Solvent medium used	Regression equation	Correlation coefficient	RSD %	Linear range (μg/ml)
1.	30:13.6:11.8% *w/v* blend urea:sodium acetate:sodium citrate	y = 0.0061x + 0.0359	0.9998	0.34-9.45	20-120

*(n=6)

concentration range of 20-120 μg/ml was chosen as the most suitable of all measurement for this method in UV spectrophotometry. Beer's law was obeyed over this concentration range.

Solubility in hydrotropic blend, spectrophotometric analysis of frusemide tablets and niosomes

By performing the solubility studies, it was found that there was negligible effect on solubility of frusemide in buffer of pH 4.0 and 8.2. Also there were significant enhancements in aqueous solubility of frusemide in urea, sodium acetate and sodium citrate solutions respectively, compared to its solubility in water. This is attributed to hydrotropy which is a solubilization phenomenon whereby addition of large amounts of a second solute results in an increase in the aqueous solubility of another solute (Poochikian and Cradock, 1979; Darwish *et al* 1989; Rasool *et al* 1991; Jain *et al* 1990). Enhancement in aqueous solubility of frusemide in blend of hydrotropic agents (30% *w/v* urea:13.6% *w/v* sodium acetate:11.8% *w/v* sodium citrate) was more than 15 fold compared to its solubility in water and was maximum as compared to individual hydrotropes and other hydrotropic blend solutions. This was attributed to mixed hydrotropic effect of hydrotropes used. As the name indicates, mixed hydrotropy includes a solubilization effect due to synergistic effect of mixture of different hydrotropic agents resulting in increased aqueous solubility under normal atmospheric conditions (Maliwal *et al* 2008; Maheshwari 2007; 2009). At higher concentrations, hydrotropic agents self associate to form the non-covalent assemblies of lower polarity.

Furthermore, the selected hydrotropic agents have a shorter hydrophobic segment, leading to higher water solubility. Therefore, this blend was used to extract out frusemide from fine powder of tablet formulation as well as niosomes. The percent label claim analyzed for two brands of frusemide was found to be 98.93±0.821% and 101.46±0.310 in case of I.P. method and was 98.85±1.345% and 100.70±0.885% in case of proposed method (**Table 2**). Percentage recovery values ranged from 99.08±1.551 to 100.81±1.211 for one formulation and 100.44±1.111 to 101.02±0.772 for other formulation (**Table 3**). Drug content in extract of hydrotropic solution was nearly same and without precipitation during 24 h.

Niosomes showed average vesicular size of 6.5±0.25 μm. The solvent 0.1 M NaOH was unable to extract entrapped frusemide from niosomes and hence did not detect drug. The drug content was uniform in repeated samples with 30:13.6:11.8% *w/v* blend of urea:sodium acetate:sodium citrate solution used as solvent with low SD and CV<2 (**Table 4**).

Table 2. Results of analysis of frusemide tablets with statistical evaluation*

Tablet Formulation	Label claim per tablet	Method of analysis	Percent drug estimated (mean±SD)	%CV	Standard error	t-test calculated t-value (t_c)
I	40 mg	I.P.	98.93%±0.821	0.536	0.598	2.564
II	40 mg	I.P.	101.46%±0.310	0.745	0.743	2.756
III	40 mg	Proposed	98.85%±1.345	1.361	0.777	2.229
IV	40 mg	Proposed	100.70%±0.855	0.849	0.494	2.315

*(n=3)

Table 3. Results of recovery studies with statistical evaluation*

Tablet Formulation	Drug present in pre-analyzed powder	Pure drug added (mg)	Percent recovery estimated (mean±SD)	%CV	Standard error	t-test calculated t-value (t_c)
I	50 mg	10 mg	99.08±1.551	1.565	0.895	3.675
II	50 mg	20 mg	100.87±1.211	1.201	0.699	3.564
III	50 mg	10 mg	100.44±1.111	1.106	0.641	3.192
IV	50 mg	20 mg	101.02±0.772	0.764	0.446	3.473

*(n=3)

Table 4. Results of analysis of entrapment efficiency of frusemide niosomes with statistical evaluation*

Niosome Formulation	Solvent of analysis	Percent entrapment efficiency (mean±SD)	%CV	Standard error	t-test calculated t-value (t_c)
I	0.1 M NaOH	Unable to detect drug	–	–	–
I	30:13.6:11.8% *w/v* blend of urea:sodium acetate:sodium citrate	92 ±0.310%	0.653	0.529	2.312

*(n=3)

Method validation and statistical analysis

Statistical comparison of the results was performed with regard to precision and accuracy using student's t-test at 95% confidence level (**Tables 2-4**). The student's t-test showed that there was no significant difference between the I.P. method and proposed method of analysis, so proposed method can be used as an analytical method in comparison to I.P. method. Also statistical evaluation of obtained data was expressed as the mean percentage of recovery, % coefficient of variation (%CV) and standard error, as shown in **Table 3**. These statistical parameters were calculated in each concentration level for the sensitivity of the method. Also in the proposed UV method for frusemide detection in niosomes, the R.S.D. values indicated satisfactory intra-day (R.S.D. of 0.78%) and inter-day variability (R.S.D. of 0.31%). A good accuracy of the method was verified with a mean recovery of 99.18% (**Table 5**). As per ANOVA results, there are linear regression ($F_{calculated}>F_{critical}$; P=0.01) and no deviation from linearity ($F_{calculated}<F_{critical}$; P=0.01).

Table 5. Experimental values obtained in recovery test for frusemide in niosomes by proposed method

Niosome Formulation used	Sample concentration (μg/ml)	Concentration of added Standard (μg/ml)	% Recovery±R.S.D. (%)
I	400	400	100.16±1.25
I	400	800	101.39±1.13
I	400	1200	101.01±1.04

CONCLUSION

The proposed method employing mixed hydrotropy concept was successfully utilized for the quantitative determination of frusemide in tablets as well as niosomes. The developed method was also satisfactorily validated as per ICH guidelines and was found to be simple, accurate, sensitive, reproducible as well as eco-friendly and cost effective with use of cheap, non-corrosive analytes for the determination of the concentration of frusemide in tablets when compared with pharmacopoeial method for the routine determination. Also, the UV method developed for the determination of frusemide in niosomes was found to be simple, rapid, precise, accurate and sensitive. Therefore, it was concluded that proposed method using mixed hydrotropy approach can be used for the drug content and entrapment efficiency analysis of both conventional tablet dosage form and TDDS.

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