International Journal of Advances in Pharmaceutical Analysis
IJAPA Vol. 4 Issue 3 (2014) 105-112

Statistically Comparable UV-spectrophotometric Method development for the Estimation of Probenecid in tablet Dosage Form with the Application of Hydrotropic Solubilization Phenomenon.

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Abstract

Statistically comparable method has been developed, validated, for estimating probenecid in tablet dosage form with the application of hydrotropic solubilization phenomenon. This research effort utilizes 2M sodium acetate as hydrotropic solubilizing agent which enhances the aqueous solubility of probenecid by 85 folds. The drug stability in hydrotropic solution was found to be more than 48hrs.The probenecid hydrotrope obeys the Beer's – Lambert law at maximum wavelength of 244.5nm. The drug response was found to be linear in the range of 2 – 26 µg/ml with correlation coefficient of 0.998.The non-interference of hydrotropic agent and additives in the course of probenecid estimation ensures the specificity of the proposed method. The mean recovery of 99.43% reflects its accuracy, obtained in good terms. The method precision results were found to be within limit expressed in %RSD (NMT 2%). The results of comparison of proposed method with standard method (IP 2010) were found to be in the similar range. The comparison provides evidence of proposed method to be simple, less time consuming, safe and eco-friendly. The validation (as per ICH 2005) of the proposed method accesses its use in the routinely quality control of probenecid in tablet dosage form.
Keywords: Probenecid, Validation, Hydrotropic agent, UV- spectroscopy.

1. Introduction

In the field of drug development, poor aqueous solubility of hydrophobic drugs has become one of the important issues especially when the drug discovery has moved from wet chemistry to combinatorial chemistry and high throughput screening. The invent of new chemical entities pave quantity of rewards but poses their own limitations in the field of analysis. In view of the fact that low solubility can hinder the development, number of organic solvents such as DMSO, DMF, ethanol, methanol, chloroform, benzene and acetonitrile have been employed for the solubilization of hydrophobic drugs to carry out spectrophotometric analysis. These organic co-solvents throw up their own toxicological liabilities on the user. Thus there is a need for finding powerful solubilizing systems that are also suitable for a wide range of poorly water soluble drugs.

Get to the bottom of solubility issue, hydrotropy is considered to be the safest technique among various techniques employed for enhancing solubility. Hydrotropes are a diverse class of water – soluble compounds that, at high concentrations, enhance water solubilities of poorly soluble solutes[1,2]. Hydrotropes have the property of self-association to form non-covalent assemblies of non-polar domains, to solubilize hydrophobic solutes at the minimal hydrotrope concentration[3-5]. Some examples of hydrotropes are nicotinamide and its derivatives, sodium salicylate, sodium benzoate, 2.0 M Sodium acetate solution, and sodium citrate which are commonly utilized to increase the water solubility of drug [6-20]. In present research work, our main focus is on simplifying the estimation of probenecid drug content in formulation on an advantage of safety and less time consumption.

Probenecid, chemically is 4-[(dipropylamino)sulfonyl]-p-(Dipropylsulfamoyl) benzoic acid widely used in the treatment of gout and is completely insoluble in water[21]. It is available in combination with ampicillin, cephalexin and cefuroxime axetil. Literature consideration reveals few assay method developments for probenecid as single drug while handful in combination with other drug molecules taking account of UV spectrophotometry, Derivative spectrophotometry, spectrophotofluorometric [22-26]. However no assay method development was found to estimate probenecid in dosage form without using organic solvents. Thus, accordingly, the need was felt to develop a simple, safe, accurate, eco- friendly, cost effective, sensitive spectrophotometric method for estimation of probenecid in dosage form by using aqueous solution of 2M sodium acetate solution, as a hydrotropic agent.

2. Material and Methods

2.1 Material and Instrumentation

Probenecid was obtained as generous gift sample from Shreeji Pharmaceutical Ltd., Mumbai, India and marketed formulation (Benemid) was purchased from local market. Sodium acetate, Hydrochloric acid, ethanol 95%, sodium hydroxide used in the study were of analytical grade obtained from Qualigens Fine Chemical, Mumbai, India.

Shimadzu UV-visible spectrophotometer (model UV-1700 series), having double beam

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detector configuration with 1 cm matched quarts cells was used.

2.2 Preliminary Solubility Studies/Saturation Solubility Studies

Solubility of probenecid was determined at 28±2°C. An excess amount of drug was added to 25 ml volumetric flasks containing 15ml of different aqueous systems viz. distilled water, sodium benzoate (1,2,4,6,8M), 2.0 M Sodium acetate solution (1,2,4,6,8 10 M) and urea (1,2,4,6,8M) solution. Enhancement of solubility of drug was increased by 85 folds in 2M sodium acetate (as compared its solubility in distilled water. The solubility enhancement ratio was determined by the following formula:

Enhancement ratio = Solubility of drug in hydrotropic solution/Solubility of drug in distilled water (gm/ml) ….. (i)

2.3 Preparation of Stock Solution

Probenecid drug sample about 50 mg was weighed accurately and transferred into 50 ml volumetric flask containing 40 ml of 2M sodium acetate solution, shaken for 5 min and diluted up to 50 ml with distilled water . The mixture was filtered through Whatmann filter paper no.1 and 5 ml of filtered solution was further diluted to 50ml with distilled water to prepare stock solution (100µg/ml).

2.4 Analytical characteristics of the probenecid hydrotrope for proposed method

The fresh aliquot of 25µg/ml was prepared from stock solution and scanned in the spectrum mode from 200 nm – 400 nm wavelength range on Shimadzu 1700 spectrophotometer. The different optical characteristics of probenecid hydrotrope such as absorption maxima, Beer's law limit, Molar Absorptivity, Sandle's sensitivity, Absorptivity ($A_{1\%, 1cm}$) were calculated. The regression analysis using the method of least squares was made for the slope (m), intercept (c) and correlation coefficient (r^2) obtained from different concentrations. The drug content was calculated by using absorptivity values obtained.

2.5 Method Validation

The proposed method was validated in accordance of ICH (2005) and USP guidelines (2004) for validation of analytical procedures in order to substantiate linearity and range, precision, recovery, robustness, LOD and LOQ for each method [27- 28].

2.6 Comparison of developed method with standard method (as per IP 2010)

2.6.1 Estimation of probenecid tablet formulation by Proposed Method:

Twenty tablets of probenecid (Benemid) were weighed and ground to fine powder. An accurately weighed powder sample equivalent to 0.100 gm of probenecid was transferred in 100ml volumetric flask and 80 ml of 2M sodium acetate solution was added the flask, shaken for about 5 min. and the volume was made up to the mark with distilled water. The solution was filtered through Whatmann filter paper no.1.The filtrate was divided into two parts A and B. Part A was kept at room temperature for 48 hrs. to check its chemical stability and precipitation, if any. Part B was diluted sufficiently with distilled water to produce stock solution of 100µg/ml. This stock solution was further diluted with distilled water to obtain final dilution of concentration of linearity range. The drug content of tablet formulation was calculated. After 48 hrs. part A solution was also analyzed in the same way as part B solution.

2.6.2 Estimation of probenecid tablet formulation (Standard Method IP 2010):

Following spectroscopic assay method given in Indian Pharmacopoeia (2010) was followed for comparison. In this method powdered tablet equivalent to 0.2 gm of probenecid was accurately weighed and dissolved in 200 ml of ethanol (95%) with addition of 5ml of 1M HCl. The mixture was heated for 30 min at 70^0C, cooled and sufficient ethanol (95%) was added to produce 250 ml and filtered. To 5 ml of this filtrate, 5 ml of 0.1M HCl was added and diluted to 250 ml again with ethanol (95%). The absorbance of this diluted solution was measured at 248.0 nm taking 332 as specific absorbance.

3. Results

3.1 Optimization of hydrotrope

Probenecid, being insoluble in water, was selected for the application of hydrotropy phenomenon. The chemical structure of probenecid is shown in fig. 1 revealing the hydrophobic property of drug with few numbers of chromophores in it. After assessing the solubility pattern as shown in fig.2, 2M sodium acetate was selected as working hydrotropic solubilizing agent for analysis.

Fig.1: Chemical structure of Probenecid

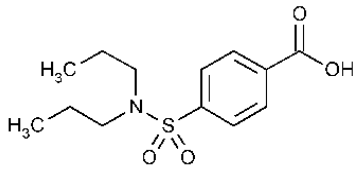
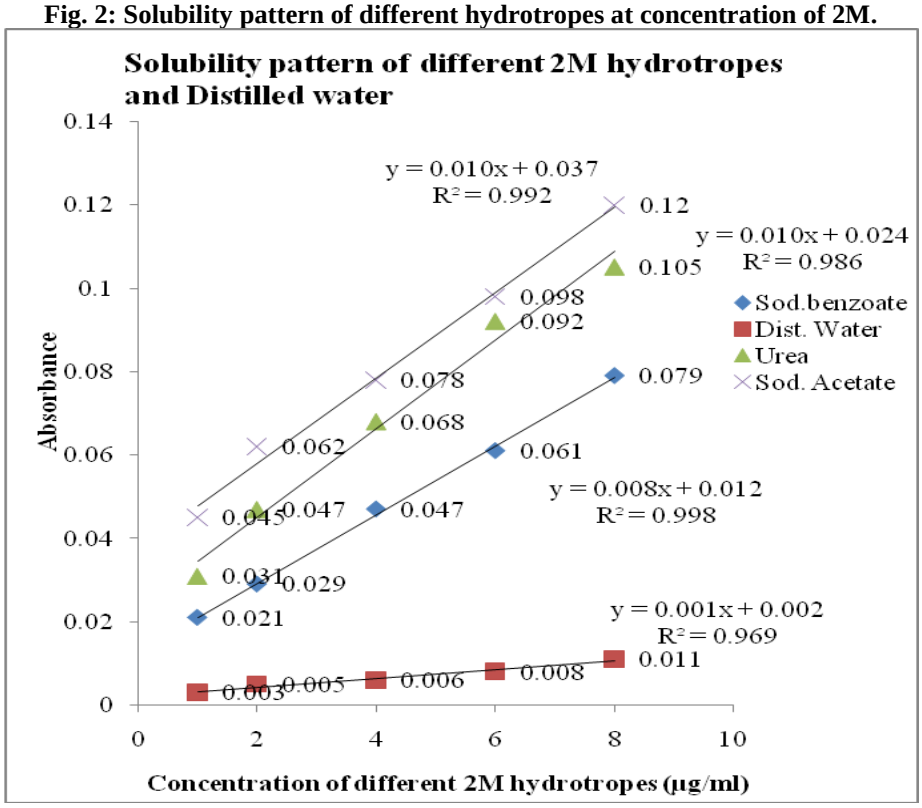


Fig. 2: Solubility pattern of different hydrotropes at concentration of 2M.



3.2 UV spectral studies

Sodium acetate 2M does not show any absorbance above 220 nm (fig.3).The other excipients (starch) in composition do not show any absorbance in analyzing range of probenecid (fig.4). Thus confirms the non-interference of hydrotropic agent and excipients in the estimation procedure.

Fig.3: Scanned spectra of 2M sodium acetate solution

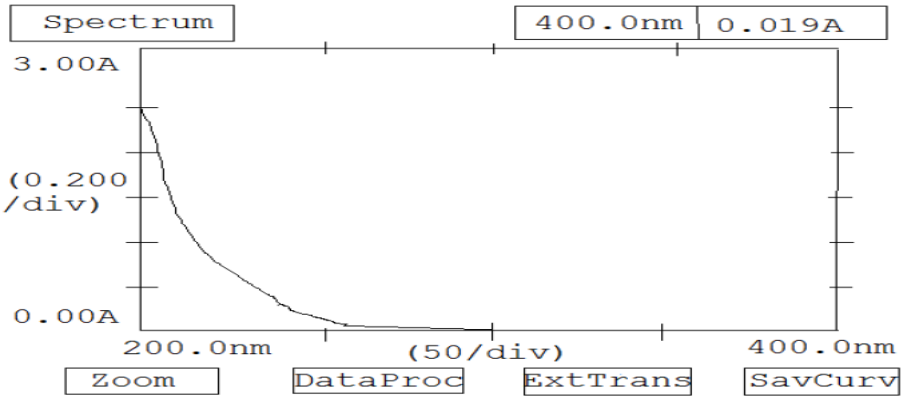
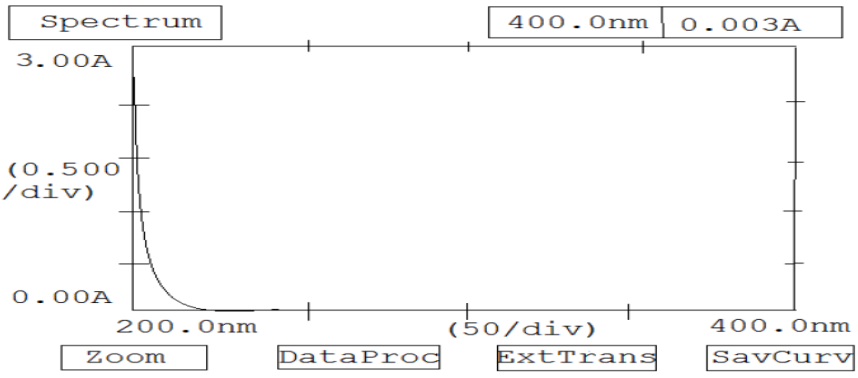


Fig.4: Scanned spectra of Starch in 2M sodium acetate.



3.3 Optical parameters of probenecid hydrotrope

The different optical characteristics of probenecid hydrotrope were calculated for proposed method and results are mentioned in Table-1.

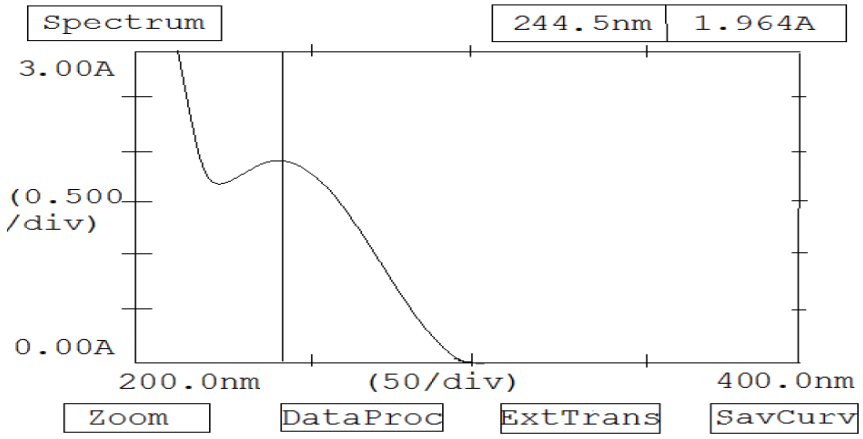
On scanning, maximum absorbance was observed at 244.5 nm and hence 244.5 nm was selected as working wavelength (Fig.5). Calibration curve was plotted between concentration verses absorbance shows obeying the Beer's – Lamberts law in the range of 2 – 26µg/ml. Absorptivity of 376 was calculated from average of five concentrations against distilled water as blank. Drug content was calculated as per the following Beer's – Lambert equation[29]:

$$A = a\ b\ c \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(ii)}$$

Table 1: Optical parameters of hydrotrope probenecid for proposed methods

S.No.	Parameters	Direct spectrophotometry
1.	Wavelength (λ)nm	244.5 nm
2.	Beer's law limit (µg/ml)	2 – 26 mcg/ml
3.	Molar absorptivity (L/mol.cm)	10729.54
4.	Sandel's sensitivity (µg/cm^2/0.001 absorbance unit)	0.026596
5.	Regression equation	y = 0.038x – 0.017
6.	Slope (m) Standard Deviation for slope (average of 5 concentration)	0.038 0.001
7.	Intercept (C) Standard Deviation for Intercept (average of 5 concentration)	0.017
8.	Correlation coefficient (r^2) Standard Deviation for Correlation coefficient (average of 5 concentration)	0.998
9.	Absorptivity ($A_{1\%,1cm}$)	376

Fig. 5: Scanned spectra of Hydrotrope probenecid



3.4 Method Validation

The validation of an analytical method confirms the characteristics of the method to satisfy the requirements of the application. Under the validation study the following parameters were studied and summarized results are shown in Table 2.

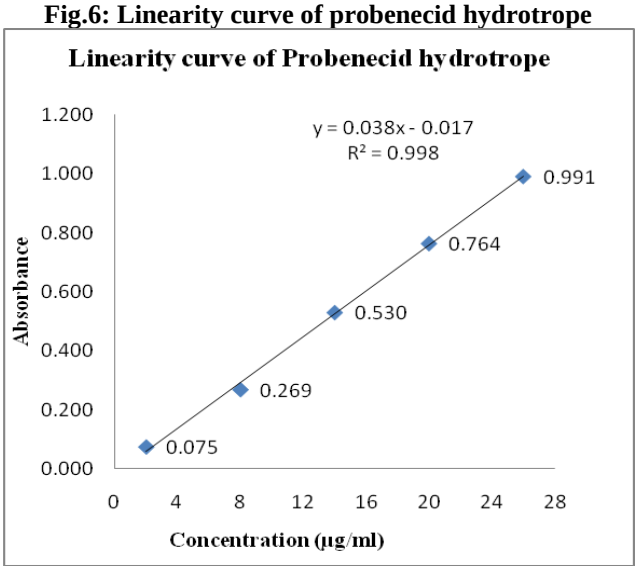
Table 2: Summary of Method Validation Parameters

S.No.	Validation parameters	Limits	Results
1.	**Linearity (r^2)**	**0.9995 – 1.000**	0.999
2.	**Range**	---------	2 – 26 µg/ml
3.	**Precision** **(Day to Day)** *Intraday* *Interday* **(Analyst to Analyst)** *Analyst I* *Analyst II*	**% RSD = NMT 2%**	 0.208% 0.100% 0.308% 0.458%
4.	**Recovery studies** *(average mean recovery)*	**98 % - 102%**	99.43%
5.	**LOB (10 replicates)** **LOD**	0.002±0.001	0.003 µg/ml 0.057 µg/ml
7.	**Robustness** *(Wavelength Decreasing & Increasing by 1 nm)*	**%Deviation = NMT 1%**	0.93% & 0.80%

3.5 Linearity

A linearity curve was plotted between concentration of probenecid hydrotrope & absorbance and found to be linear over analytical range of 2 - 26µg/ml with correlation coefficient value of 0.999 at wavelength of 244.5 nm (fig.6) against distilled water as the reagent blank.

Fig.6: Linearity curve of probenecid hydrotrope



3.6 Recovery studies

Accuracy of proposed method was ascertained on the basis of recovery studies, was performed by the standard addition method at 20%, 30%, 40% level with minimum of nine determinations over three concentration levels within specified range .The percent recoveries, standard deviations, coefficient of variation were calculated as depicted in table 3. The mean recovery was found to 99.43%.

Table 3: Results of recovery studies

Brand name	Theoretical concentration (µg/ml)	Amount added (%)	Average concentration recovered (%)	Percentage recovery (mean ± SD) (n = 9)	Coefficient of variation (%)	*Standard error
Benemid	100	----	99.73	99.73 ± 0.265	0.266	0.153
	100	20%	99.66	99.66 ± 0.713	0.715	0.412
	100	30%	99.25	99.25 ± 0.519	0.523	0.300
	100	40%	99.39	99.39 ± 0.786	0.791	0.454

* n=9

3.7 Precision

Precision was evaluated as % RSD at two different parameters as repeatability and intermediate with three concentration & three replicates. The coefficient of variation and % mean ±Standard deviation of Intraday and Interday and analyst to analyst precision for proposed method were calculated and found to be less than 2% respectively as shown in the Table 4.

Table 4: Precision study results

Validation parameter	Percentage Mean ±S.D *(n = 9)	% RSD
Intermediate Precision		
(Day to day)		0.210
Intra day	100.23 ± 0.208	0.100
Interday	99.60 ± 0.100	
(Analyst to analyst)		
Analyst I	99.29 ± 0.306	0.308
Analyst II	99.61 ± 0.456	0.458

*Mean of 9 determinations (3 replicates at 3 concentration level).

SD = Standard deviation and RSD = Relative standard deviation.

3.8 Specificity

Specificity for probenecid hydrotrope was performed by spiking the appropriate level of starch as excipients and determined the assay results. The starch concentration was found to be 0.05% - 0.06% in the assay.

3.9 LOB and LOD

LOB is the highest apparent analyte concentration expected to be found when replicates of blank sample containing no analyte are tested.It is calculated by the use of following equation:

$$LOB = mean_{blank} + 1.645(SD_{blank}) \quad \text{(iii)}$$

The LOD for probenecid hydrotrope was calculated from obtained LOB value by using the following equation:

$$LOD = LOB + 1.645(SD_{\text{low concentration sample}}) \quad \text{(iv)}$$

The value of LOB at 10 replicates with standard deviation of 0.001 was found 0.003µg/ml while LOD for Probenecid hydrotrope was calculated as 0.057µg/ml.

3.10 Robustness

The robustness was performed by making change of ± 1 nm in wavelength. The deliberate alteration of wavelength results in 0.93% & 0.80% deviation demonstrates that the developed method was robust and unaffected by minor changes. The results are given in table 2.

3.11 Comparison of proposed method with standard method (IP 2010)

The proposed method was compared with standard method as mentioned in Indian Pharmacopoeia 2010. Drug content of tablet formulation was calculated for each method. The percent estimated by proposed method was found to be 99.73% while 98.40% by standard method. The statistical evaluation of analytical data for each formulation was incorporated in table 5.

Table 5: Comparison of results of standard method and proposed method

Method → / Concentration ↓	IP method (2010)	Proposed method
	% estimated in formulation	% estimated in formulation
100 mg	98.40	100.0
	98.74	99.73
	98.07	99.47
Average.	98.40	99.73
Standard deviation	98.40±0.335	99.73±0.265
%RSD**	0.340	0.266
SEM*	0.193	0.153

*SEM – Standard Error of Mean; **RSD - Relative Standard Deviation.

4. Discussion

Results of solubility studies indicated that, enhancement in aqueous solubility of probenecid in 2M sodium acetate solution, as compared to its solubility in distilled water. Based on stability of drug in hydrotropic solution reveals that estimations can be done within 48h at least, without having any unfavorable effect on drug stability as part A solution of drug in hydrotropic solution did not show any precipitation or color change. Moreover the drug content of part A solution was same as in part B solution. Thus study divulges the drug stability in hydrotropic solution for 48 hrs.

The non-interference of hydrotropic agent and additives in the analysis range of probenecid was also uncovered by the UV spectral studies as 2M sodium acetate solution and starch did not show any absorbance above 220 nm. Thus confirms the applicability and reproducibility of the developed method.

The analytical characteristics of the proposed method for probenecid hydrotrope gave the impression of drug sensitivity towards instrument and vice-versa. The summarized results of method validation are further substantiated and authenticate the method for its intended use. The linear regression analysis driven with acceptable intercepts and correlation coefficients indicates a good correlation between concentration and absorbance within the concentration range tested for each method. Results of precision studies at different level were found to be within acceptable limits (RSD < 2.0) suggesting the method is highly precised. The mean percent recovery was found to be 99.43% which is very close to 100, indicating the accuracy of analytical method. The results of LOD and LOQ elaborate sufficient sensitivity of method. The robustness study reveals low values of percent deviation demonstrating that developed method was robust and unaffected by minor changes. Specificity of the method performed by placebo as well as spectral study of additives, both gave the impression of method specificity.

In comparing the mean percent label claims, estimated by standard method and those estimated by proposed method were found to be are very close to 100 while low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method. Thus it is evident that there is good agreement between the amounts estimated and those claimed by the manufacturers. The proposed method has an advantage of less time consuming in addition to less number of chemicals

are required over standard method. Moreover the proposed method is safe, simple as it does not requires the standardization of mineral acids or alkalis before their utilization in the analysis process.

5. Conclusion

In Nutshell, the developed method is easier to apply, safe, eco – friendly, cost – effective, sensitive and accurate precluding the use of toxic, detrimental, costlier, organic solvents. Hence it makes the quantitative analysis more interesting as well as exploring the application of hydrotropic solublization phenomenon.

Acknowledgement

The authors are gratefully acknowledged the management of ASBASJSM college of Pharmacy, Bela (Ropar), India for providing the research facility and financial support. Grant No.: PHANL/2010 – 12/103035039512.

References

1. Saleh AM, El-Khordagui LK. Hydrotropic agents: A new definition. *Int J Pharm* 1985; 24(2-3):231–238.
2. Lee J, Lee SC, Acharya G, Chang CJ, Park K. Hydrotropic solubilization of paclitaxel: Analysis of chemical structures for hydrotropic property. *Pharm Res* 2003; 20:1022–1030.
3. Kumar ST, Gandhi NN. (2012). Association model of hydrotropy for the effect of hydrotropes on solubility and mass transfer coefficient of acetylsalicylic acid. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012; 4:600 – 605.
4. Desai M., Parikh J. Thermodynamic study for aggregation behavior of hydrotropic solution. *World Academy of Science, Engineering and Technology* 2009; 57:227-229.
5. Ooya T, Huh KM, Saitoh M, Tamiya E, Park K. Self-assembly of cholesterol-hydrotropic dendrimer conjugates into micelle-like structure: Preparation and hydrotropic solubilization of paclitaxel. *Science and Technology of Advanced Materials* 2005; 6:452 – 456.
6. Jain N, Jain R, Jain A , Pandey SP, Jain D.K. Spectrophotometric method development and validation for quantitative estimation of amlodipine besylate in bulk drug and their dosage forms by using hydrotropic agent. *Eurasian J. Anal.Chem* 2010; 5: 212-217.
7. Pandey S, Maheshwari RK. A Novel Spectroscopic Method for the estimation of Ketoprofen in tablet dosage form using Hydrotropic Solubilization Phenomenon. *World Applied Sciences Journal* 2010; 11:1524 – 1527.
8. Maheshwari R.K., Indurkhya A. Novel application of mixed hydrotropic solubilization technique in the formulation and evaluation of hydrotropic solid dispersion of aceclofenac. Asian *journal of pharmaceutics* 2010; 4: 235 – 238.
9. Vemula VR, Lagishetty V, Lingala S.Solubility Enhancement techniques. *International Journal of Pharmaceutical Sciences Review and Research 2010;* 5, 41-51.
10. Saravanakumar G, Choi KY, Yoon HY, Kim K, Park JH, Kwon IC, Park K. Hydrotropic hyaluronic acid conjugates: Synthesis, characterization, and implications as a carrier of paclitaxel. *International Journal of Pharmaceutics* 2010; 394: 154 - 161.
11. Maheshwari R.K., Lakkadwala S, Vyas R, Ghode P. Spectrophotometric determination of naproxen tablets using niacinamide as hydrotropic solubilizing additive. *Journal of Current Pharmaceutical Research* 2010; 4: 11 – 14.
12. Maheshwari RK, Bishnoi SR, Kumar D, Murali K. Quantitative spectrophotometric determination of ornidazole tablet formulations using ibuprofen sodium as hydrotropic solubilizing agent. *Digest Journal of Nanomaterials and Biostructures* 2010; 5: 97-100.
13. Maheswari RK, Chandrawashi H, Gupta N. Quantitative estimation of aspirin in tablets and bulk sample using metformin hydrochloride as hydrotropic agent. *International Journal of Pharmacy and Pharmaceutical Sciences* 2010; 2: 21-23.
14. Maheshwari RK, Archana A, Amit R, Megha A. Eco-friendly spectrophotometic estimation of atenolol tablets using metformin hydrochloride as hydrotropic solubilizing agent. Journal of Global Pharma Technology 2010; 2: 93-96.
15. Jayakumar, C., Kumar, D.K., Nesakumar, D., Gandhi, N.N. Quantitative analysis of theophylline bulk sample using sodium salicylate hydrotrope. *International Journal of Pharmacy and Pharmaceutical Sciences* 2010; 2: 124-125.
16. Sherje AP, Anand R, Sriram W, Vanshiv SD. Mixed hydrotropy in spectrophotometric analysis of nitazoxanide. *International Journal of Chem Tech Research* 2010; 2: 1966-1969.
17. Maheshwari R K, Rajput M, Sinha S. New quantitative estimation of benzoic acid bulk sample using calcium disodium-edetate as hydrotropic solubilizing agent. *Asian Journal of Pharmaceutical and Clinical Research* 2010; 3:43-45.
18. Sharma MC, Sharma S. spectrophotometric determination and application of hydrotropic solubilization in the quantitative analysis of ranitidine hydrochloride in pharmaceutical dosage form. *International Journal of Pharm Tech Research* 2011; 3:253-255.
19. Maheshwari RK, Bishnoi SR, Kumar D. Spectrophotometric analysis of hydrochlorothiazide tablets using chlorpheniramine maleate as hydrotropic solubilizing agent. *Asian Journal of Chemistry* 2008; 20: 6594-6596.
20. Jain, A.K. Solubilization of indomethacin using hydrotropes for aqueous injection. *European*

Journal of Pharmaceutics and Biopharmaceutics 2008; 68:701-714.
21. Indian Pharmacopoeia, Government of India ministry of health and family Welfare. 4thed. The Controller of Publication, Delhi, 2010;1965.
22. Kurian T, Kurien J. Simultaneous multicomponent spectrophotometric analysis of ampicillin and probenecid in pharmaceutical formulation by derivative spectroscopy. *HYGEIA: Journal for drugs and medicines* 2011; 3: 57 – 61.
23. Chaudhari SV, Karnik A, Adhikary A, Tandale RS, Vavia PR. Simultaneous UV spectrophotometric method for the estimation of cefuroxime axetil and probenecid from solid dosage forms. *Indian Journal of Pharmaceutical Sciences* 2008; 68: 59.
24. Jain D, Jain DK, Trivedi P. Derivative spectrophotometric method for simultaneous estimation of cephalexin and probenecid in two component solid dosage form. *Indian Journal of Pharmaceutical Sciences* 1997; 59: 324 – 327.
25. Shinde VM**,** Desai BS**,** Tendolkar NM. Simultaneous determination of cephalexin & probenecid from tablets by RP-HPLC. *Indian Journal of Pharmaceutical sciences* 1994; 56: 58 – 60.
26. Vollmer PJ, Chastonay R, Haneke C, Kantor N, Blyth R. High pressure liquid chromatographic method for probenecid in flavored oral suspensions containing ampicillin: collaborative studies. *Journal - Association Official Analytical Chemists* 1977; 60:1345 – 9.
27. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Tripartite Guidelines (ICH 2005) Q2A and Q2B.
28. USP, The official Compendia of Standards. Asian edition 2004; 1225 – 1226.
29. Beckett, A. H., Stenlake, J.B. (2007) in *Practical pharmaceutical chemistry*, Vol.2, Davidson, A.G., CBS Publishers, New Delhi, pp 267.