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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MEMANTINE AND DONEPEZIL IN MARKETED FORMULATION USING HYDROTROPY PHENOMENA

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ABSTRACT

"Hydrotropy" has been used to designate the increase in solubility of various substances due to the presence of large amounts of additives. Various hydrotropic agents such as sodium salicylate, sodium benzoate, urea, nicotinamide, sodium citrate and sodium acetate have been used to enhance the aqueous solubility of a large number of drugs. The hydrotropes are a special class of compounds that exhibit distinct solution properties. They may self-associate in aqueous medium, comparable to amphiphile self-association or micellization. They are efficient solubilizers and can influence the formation of micelle and micro emulsion. Using hydrotropic agents is one of the easiest ways of increasing water solubility of poorly soluble drugs, since it only requires mixing the drugs with the hydrotrope in water. The hydrotrope approach does not require chemical modification of hydrophobic drugs, use of organic solvents, or preparation of emulsion systems. Considerable research has focused on attaching drugs to polymers in order to improve pharmacokinetic properties.

Keywords: Hydrotropy, Aqueous solubility, amphiphile, micellization, solubilizers, micro emulsion, pharmacokinetic properties

INTRODUCTION:

Hydrotropes are water-soluble, surface-active compounds which can significantly affect the solubility of poorly soluble drugs, most likely due to the formation of organized assemblies within solution. [1] The issues encountered with traditional surfactant solubilization, such as emulsification, are not observed for hydrotropes due to the small hydrophobic part of the molecule. Considerable research has focused on attaching drugs to

polymers in order to improve pharmacokinetic properties. [2,3]

Various techniques have been employed to enhance the aqueous solubility of poorly water-soluble drugs. Hydrotropic solubilization is one of them. In the hydrotropic solubilization phenomenon, addition of large amount of second solute results in an increase in the aqueous solubility of another solute. Concentrated aqueous hydrotropic solutions of urea, nicotinamide, sodium benzoate, sodium salicylate, sodium acetate and sodium citrate have been observed to enhance the aqueous solubility of poorly water-soluble drugs. [4]

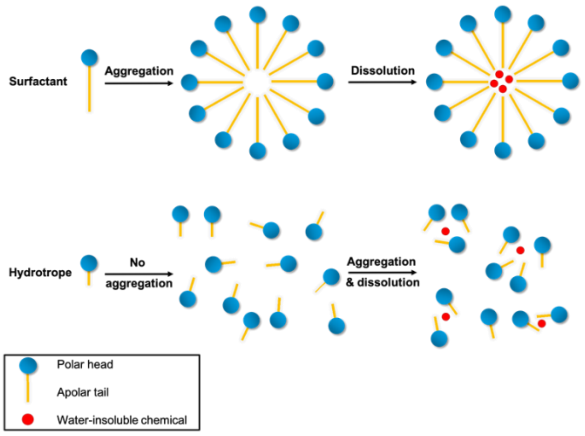

Fig. 1: Influences of Hydrotrope

Methods to enhance the solubility of a drug: [5]

Following approaches can be employed to enhance the aqueous solubility of a drug solute.

1. Alteration of the pH of the solvent
2. Using co-solvents
3. Effect of dielectric constant
4. Use of surface-active agents
5. Complexation
6. Hydrotropic solubilization
7. Chemical modification of the drug

Pharmaceutical applications: [6]

1. Preparation of aqueous solutions of poorly soluble drugs for in vitro experiments and in vivo animal experiments.
2. The poor water solubilities of many drugs and drug candidates make it difficult to do experiments for identifying bio efficacy and dose-response studies.
3. The hydrotrope approach does not require chemical modification of hydrophobic drugs, use of organic solvents, or preparation of emulsion systems.
4. The resulting concentration of the substance in water is effectively greater in the presence of hydrotropic agent than in its absence.

Analysis of drug utilized the organic solvent which are costlier, toxic and causing environment pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents so that a simple, accurate, novel, safe and precise method has been developed for estimation of poorly water-soluble drugs.

Donepezil is postulated to exert its therapeutic effect by enhancing cholinergic function and acetylcholine levels of the brain. This is accomplished by increasing the concentration of acetylcholine through reversible inhibition of its hydrolysis by acetyl cholinesterase. The recommended initial dose of donepezil is 5 mg taken once daily. A 50% inhibition of acetylcholinesterase activity is obtained at a plasma drug concentration of 15.6 ng/ml, and the inhibition plateaus at the plasma concentration of donepezil higher than 50 ng/ml. [7,8]

Memantine hydrochloride (1-amino3, 5-dimethyladamantane hydrochloride) is a tricyclic amine chemically and pharmacologically related to the antiviral prototype amantadine and its α-methyl derivative rimantadine. Amantadine and rimantadine have been approved in the U.S. for the prophylaxis and treatment of influenza. Amantadine is also approved for the treatment of Parkinsonism. Memantine is used in Parkinson's disease and movement disorders. [8]

Memantine and donepezil combination is used to treat dementia (memory loss and mental changes) associated with moderate or severe Alzheimer's disease. Various organic solvents such as methanol, chloroform, dimethyl formamide and acetonitrile have been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents. [9]

EXPERIMENTAL WORK AND RESULTS:

1. **Melting point:** The melting point of Memantine and Donepezil was found to be 258-260°C and 220-222°C respectively by the open capillary methods. The melting point of drug was recorded and compared with reported values.
2. **Solubility:** Solubility of MNT and DPZ was determined at 25±1°C. Accurately weighed 10 mg MNT and DPZ was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solutions were filtered through Whatman filter paper No. 41. The filtrates were diluted suitably and analysed spectrophotometrically against solvent. Enhancement of solubility was more than 80 and 90% for MNT and DPZ respectively in hydrotropic solution. The enhancement of solubility of MNT and DPZ due to the hydrotropic solubilization phenomenon. Results of solubility in different solvent.

Table. 1: Solubility of Drug in Different Solvents

S. No.	Solvents	SOLUBILITY	
		MNT	DPZ
1	Water	-	-
2	Hot water	-	-
3	Cold water	-	-

4	2M Sodium acetate	-	-
5	8M Urea	-	-
6	2M Sodium Citrate	-	+
7	2M Sodium Benzoate	+	+
8	2M Sodium acetate	-	-
9	2M Sodium Benzoate: 2M Sodium acetate (1:1v/v)	+	+

3. **Calibration curve:** The λmax was determined by employing following method both drugs was accurately weighed and dissolved in water in 10 ml volumetric flasks. 0.1ml of solution was withdrawn and then diluted up to 10ml resulting in a solution of strength 10µg/ml. then this solution was scan from 200-400 nm in Spectrophotometer (Lab India 3000+) against the reagent blank.
4. **Preparation of Working Standard Solution**

a. 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml from sub stock solution (Stock-B) were taken separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This provided the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for DPZ

b. Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with RO Water. This gave the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml respectively for MNT.

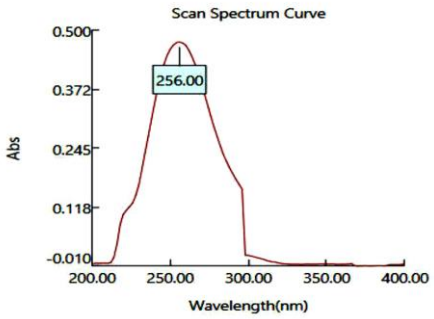

Fig.2: Determination of λmax of MNT

Table. 2: Linearity of MNT

Standard Conc. (µg/ml)	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Mean
0	0	0	0	0	0	0
5	0.142	0.143	0.145	0.144	0.145	0.144
10	0.283	0.285	0.286	0.285	0.285	0.285
15	0.424	0.424	0.425	0.424	0.425	0.424
20	0.561	0.563	0.562	0.563	0.562	0.562
25	0.703	0.704	0.707	0.706	0.706	0.705

Correlation Coefficient (r2)						0.999
Slope (m)						0.028
Intercept (c)						0.002

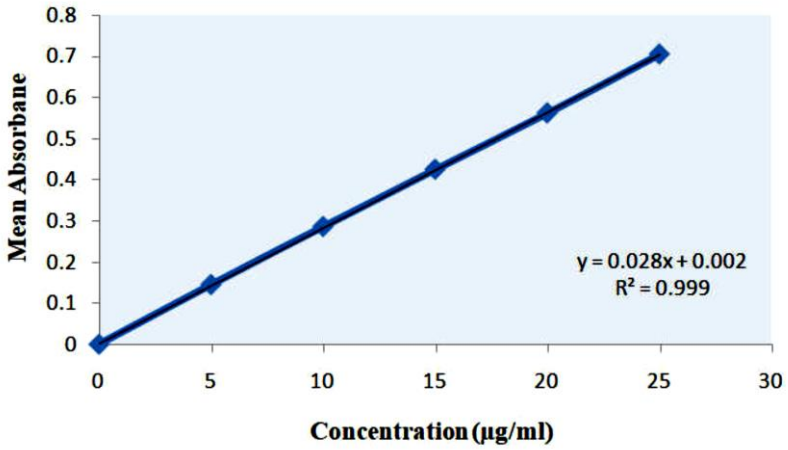

Fig.3: Calibration Curve of MNT

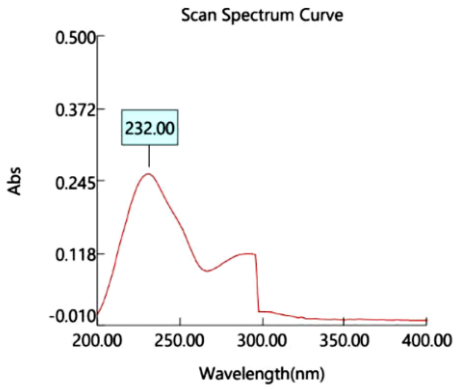

Fig.4: Determination of λmax of DPZ

Table. 3: Linearity of DPZ

Standard Conc. (µg/ml)	**Rep-1**	**Rep-2**	**Rep-3**	**Rep-4**	**Rep-5**	**Mean**
0	0	0	0	0	0	0
5	0.125	0.128	0.126	0.125	0.127	0.126
10	0.249	0.248	0.249	0.247	0.248	0.248
15	0.365	0.364	0.366	0.365	0.363	0.365
20	0.482	0.483	0.483	0.485	0.483	0.483
25	0.605	0.604	0.605	0.603	0.604	0.604
Correlation Coefficient (r2)						0.999
Slope (m)						0.024
Intercept (c)						0.003

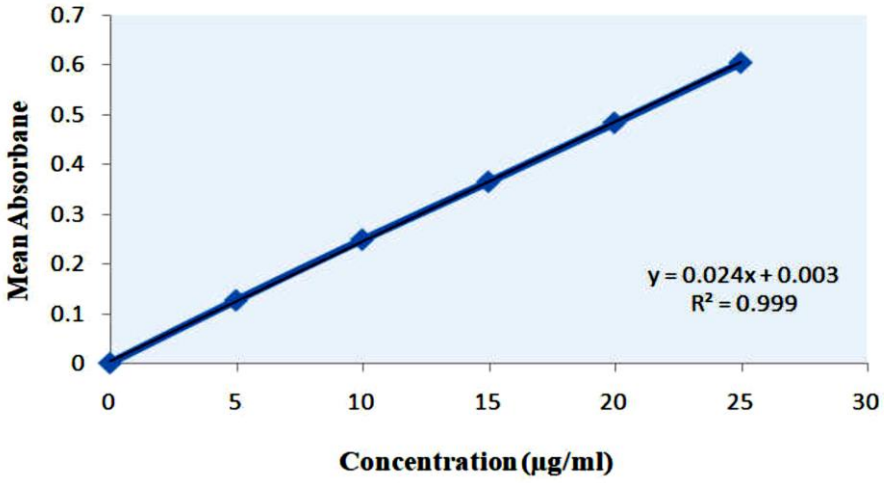

Fig.5: Calibration Curve of DPZ

Mixed Standard Study:

Tablets of MNT and DPZ combination are available in 5:10. Mixed standard are prepared in the ratio of 5:10 from standard sub stock solution of 2-10 µg /ml and 5-25µg/ml in 3 replicates of 5 concentrations. Solutions containing known concentration of two drugs are considered as laboratory samples (mix standards) to check the results of developed method.

Table. 5: Mixed Standard Study of MNT and DPZ

Conc. Present (µg/ml)		Replicate-1				Replicate-2				Replicate-3			
		Conc. Found (µg/ml)		% Conc. Found		Conc. Found (µg/ml)		% Conc. Found		Conc. Found (µg/ml)		% Conc. Found	
MNT	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**
5	**5**	4.95	4.93	99.00	98.60	4.85	4.85	97.00	97.00	4.85	4.98	97.00	99.6
10	**10**	9.85	9.85	98.50	98.50	9.65	9.78	96.50	97.80	9.85	9.85	98.50	98.5
15	**15**	14.85	14.96	99.00	99.73	14.96	14.85	99.73	99.00	14.96	14.82	99.73	98.8
20	**20**	19.96	19.95	99.80	99.75	19.85	19.82	99.25	99.10	19.98	19.96	99.90	99.8
25	**25**	24.68	24.96	98.72	99.84	24.92	24.68	99.68	98.72	24.78	24.78	99.12	99.12
										MEAN*		**98.85**	**99.16**
										SD*		**1.173**	**0.541**
										% RSD*		**1.186**	**0.545**

* Mean of 3 replicate and 5 concentrations

Validation of simultaneous equation method

A_1: Linearity

Linearity of both drugs was established by response ratios of drugs. Response ratio of drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Table.6: Response Ratio of MNT and DPZ

S. No.	MNT			DPZ		
	Conc. (µg/ml)	ABS	Response Ratio	Conc. (µg/ml)	ABS	Response Ratio
1	0	0	0	0	0	0
2	5	0.144	0.029	5	0.126	0.025
3	10	0.285	0.029	10	0.248	0.025
4	15	0.424	0.028	15	0.365	0.024
5	20	0.562	0.028	20	0.483	0.024
6	25	0.705	0.028	25	0.604	0.024

B1: Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of MNT and DPZ to reanalysed tablet solutions.

Table. 7: Recovery study of MNT (100% level)

MNT tablet (□g/ml	Std. MNT Added (□g/ml)	Rep-1		Rep-2		Rep-3		MNT % Mean
		MNT Found	% Found	MNT Found	% Found	MNT Found	MNT Found	
5	5	4.95	99.00	4.85	97.00	4.99	99.80	98.60
10	10	9.95	99.50	9.85	98.50	9.98	99.80	99.27
15	15	14.78	98.53	14.78	98.53	14.96	99.73	98.93
20	20	19.96	99.80	19.96	99.80	19.98	99.90	99.83
25	25	24.74	98.96	24.78	99.12	24.78	99.12	99.07
							MEAN*	**99.14**
							SD*	**0.457**
							% RSD*	**0.461**

* Mean of 3 replicate and 5 concentrations

Table. 8: Recovery study of DPZ (100% level)

DPZ Tablet (□g/ml	Std. DPZ Added (□g/ml)	Rep-1		Rep-2		Rep-3		DPZ % Mean
		DPZ Found	% Found	DPZ Found	% Found	DPZ Found	DPZ Found	
5	5	4.98	96.00	4.96	99.20	4.99	99.80	98.33
10	10	9.96	97.75	9.98	99.80	9.96	99.60	99.05
15	15	14.85	94.00	14.78	98.53	14.85	99.00	97.18
20	20	19.96	97.75	19.96	99.80	19.98	99.90	99.15
25	25	24.95	96.40	24.85	99.40	24.78	99.12	98.31
							MEAN*	**98.40**
							SD*	**0.706**
							% RSD*	**0.717**

* Mean of 3 replicate and 5 concentrations

C1: Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility.

Table. 9: Repeatability of MNT

Replicate	Concentration Found					
	5	**10**	**15**	**20**	**25**	
Replicate-1	4.95	9.98	14.85	19.98	24.65	
Replicate-2	4.85	9.65	14.65	19.85	24.74	
Replicate-3	4.98	9.77	14.88	19.96	24.65	
Replicate-4	4.78	9.65	14.65	19.98	24.85	
Replicate-5	4.96	9.88	14.78	19.78	24.63	
Mean	4.904	9.786	14.762	19.91	24.704	
% Mean	98.08	97.86	98.4133	99.55	98.816	**98.544**
S.D.	0.086	0.145	0.108	0.091	0.092	**0.104**
% R.S.D.	0.087	0.148	0.110	0.091	0.093	**0.106**

Table. 10: Repeatability of DPZ

Replicate	Concentration Found					
	5	**10**	**15**	**20**	**25**	
Replicate-1	4.95	9.95	14.85	19.98	24.78	
Replicate-2	4.85	9.98	14.96	20.01	24.85	
Replicate-3	5.01	9.96	14.82	19.95	24.96	
Replicate-4	4.92	9.95	14.98	19.89	24.96	
Replicate-5	4.86	9.89	14.96	19.95	24.78	
Mean	4.918	9.946	14.914	19.956	24.866	
% Mean	98.36	99.46	99.43	99.78	99.46	**99.30**
S.D.	0.066	0.034	0.073	0.044	0.090	**0.062**
% R.S.D.	0.067	0.034	0.074	0.045	0.091	**0.062**

Analysis of synthetic mixture:

Mixed Blends of MNT and DPZ were weighed and ground to a fine powder; amount equal to 5mg of DPZ was taken in 10 ml volumetric flask. The present in this amount of tablet powder was 10mg MNT. Then 4ml of 2M Sodium Benzoate: 2M Sodium acetate (1:1 v/v) was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration

was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO Water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from Simultaneous Equation Method. The procedure was repeated for five times.

Table.11: Analysis of Tablet Formulation of MNT and DPZ

Conc. Present (µg/ml)		Replicate-1				Replicate-2				Replicate-3			
		Conc. Found (µg/ml)		% Conc. Found		Conc. Found (µg/ml)		% Conc. Found		Conc. Found (µg/ml)		% Conc. Found	
MNT	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**	**MNT**	**DPZ**
5	**5**	4.85	4.85	97.00	97.00	4.95	4.78	99.00	95.60	4.78	4.99	95.60	99.8
10	**10**	9.98	9.96	99.80	99.60	9.85	9.65	98.50	96.50	9.68	9.78	96.80	97.8
15	**15**	14.85	14.85	99.00	99.00	14.96	14.85	99.73	99.00	14.95	14.85	99.67	99
20	**20**	19.96	19.98	99.80	99.90	19.98	19.85	99.90	99.25	19.95	19.96	99.75	99.8
25	**25**	24.78	24.65	99.12	98.60	24.85	24.69	99.40	98.76	24.88	24.65	99.52	98.6

SUMMARY AND CONCLUSION:

Modern medicines for human use are required to comply with specific standards and regulation set forth by the concerned authorities. The efficacy and safety of medicinal products can only be assured by analytical monitoring of its quality. In the present study, a successful attempt was made for the Spectrophotometric quantitative estimation of the new antihypertensive marketed combinations by using hydrotropic agent.

It is considered as an application of procedures necessary to determine and estimate the identity, strength, quality and purity of drug. Therefore, the quality control laboratory is considered as the backbone of the Pharma industries with ever-increasing need for the development of analytical techniques for drug formulation.

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