To Develop Multi Purposed Nano Suspension for Ambroxol Hydrochloride and Doxofylline Using Basic Concept of Six Sigma Methodology Along With Novel Method for Simultaneous Determination of Active Ingredients of Newly Developed Nano Suspension

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Abstract: Poor solubility in water has become a major issue in the formulations of several chemicals, since poor solubility is often linked to low bioavailability. Nano suspensions are one formulation that may be able to aid with this problem. The present study is prone to build a multifunctional nano suspension by the use of the most frequently used antibiotic or a mucolytic agent, based on the same principle and utility of Nano suspension. The six-sigma DMADV idea for Doxofylline or Ambroxol Hydrochloride was used to create the nano suspension. According to official requirements, the created nano suspension achieves the satisfactory performance in terms of any and all formulation assessment parameters. A unique and quick spectroscopic approach has been devised for estimating active ingredients of the newly created nano solution. According to the ICH criteria for analytical method validations, the proposed technique achieves the satisfactory performance in terms of all validation parameters. The Nano suspension created may be utilized to treat a variety of respiratory ailments, gastrointestinal problems, sexually transmitted disease therapy, and sepsis prophylaxis following surgery.

Keywords: Ambroxol Hydrochloride, Analytical Method Validation, Doxofylline, Nano Suspension.

1. INTRODUCTION

Nanosuspension is indeed a colloidal dispersion of submicron-sized pharmaceutical particles. A pharmaceutical nanosuspension is indeed a watery vehicle containing finely collided, biphasic, dispersed powerful medicine particles with a size of less than 1 m which has been balanced out with surfactants or polymers as well as being ready for drug delivery using proper cycles. Finely dispersed solid medication particles suspended in water media are portrayed as pharmaceuticals nanosuspension. The demand of nanosuspension is growing these days for a variety of factors, including higher dissolvability and natural execution, simplicity of development or scale-up, long-term physical soundness, adaptability, better oral absorption, but also improved dosage proportionality [1], [2].

Nanosuspensions are a much more cost-effective alternatives to liposomes or other traditional colloidal drug carriers in terms of technical preparation. It's used to make a physiologically more stable products out of medications that aren't very water soluble. There are two approaches for making nanosuspensions: "Top-down process technology" or "Bottom-up process technology." From big particles to micro particles to nano particles, the top-down procedure includes a disintegration strategy. The bottom-up technique is a way of assembling nanoparticles from molecules [3]–[8].

Six Sigma is a process for creating and delivering near-perfect labour and goods. The main motivation behind Six Sigma is that if you can figure out how many "blemishes" there are in a cycle, you can figure out how to get rid of them and get as near to "zero flaws" as possible [9]. The primary goal of six sigma is to minimize variance, reduce defects/rework, increase yields/productivity, or improve customer satisfaction. The six-sigma process has two well-known methodologies. The first is DMAIC, while the second is DMADV. DMAIC or DMADV are acronyms for two

techniques, each of which has five steps. Define, Measure, Improve, Analyze, and Control are acronyms for DMAIC, whereas DMADV stands for Define, Analyze, Measure, Design, or Verify. DMAIC is a business process improvement technique, while DMADV is a project management tool for developing new products [10]. The present study is based on the successful application of the six-sigma DMADV technique for the formulation of a unique nanosuspension for two commonly used active medicinal components, Ambroxol Hydrochloride or Doxofylline (DOX). The study is being expanded with the development of simultaneous analysis methods for Ambroxol Hydrochloride or Doxofylline in a newly designed Nano suspension that does not need previous separation. Figure 1 and Figure 2 depict the structures of Doxofylline or Ambrol hydrochloride, accordingly.

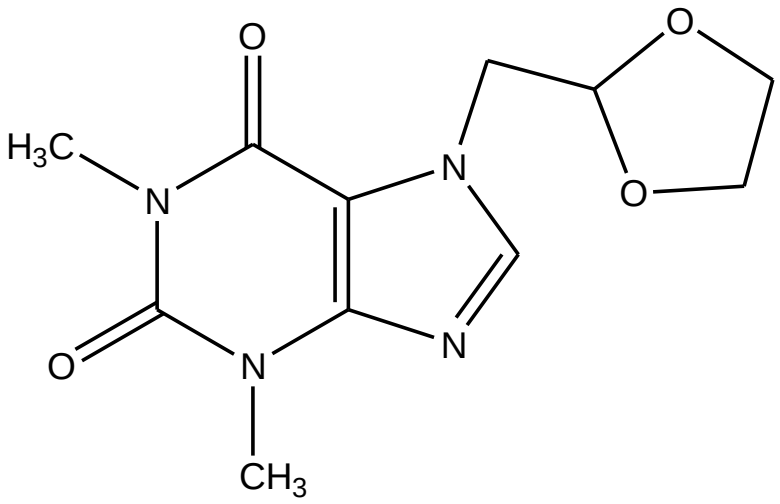

Figure 1: Structure of Doxofylline (7-[(1,3-dioxolan-2-yl)methyl]-1,3-dimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione)

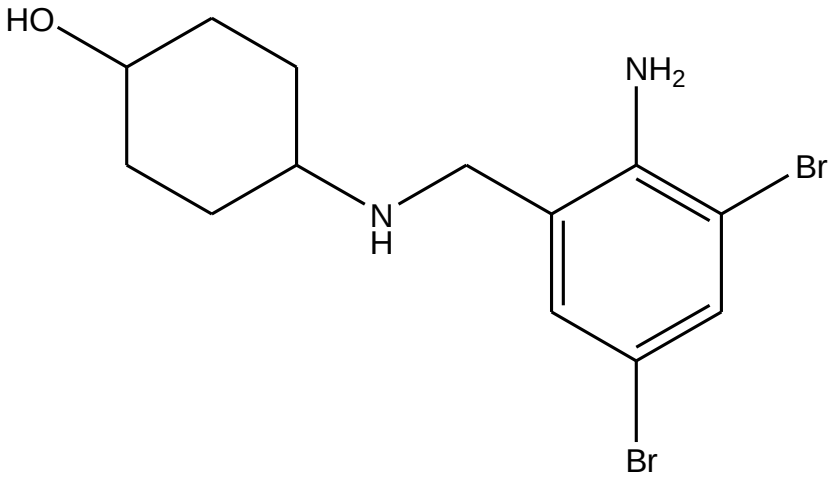

Figure 2: Structure of Ambroxol HCl (4-[(2-amino-3,5 dibromophenyl) methylamino] cyclohexan-1-ol)

2. LITERATURE REVIEW

Bernard Van Eerdenbrugh et al. investigated top-down manufacture of drug nanocrystals, including nano emulsion stability, miniaturisation, and transformations into solid products. In the last 10–15 years, medication formulation as nanocrystals has evolved into a mature drug delivery technology, with five medicines presently on the market. These systems' rapid dissolving velocity is a crucial property that allows for better absorption after oral administration. This mini-review focuses on three areas where drug nanocrystals have been used recently. The first topic is nanosuspension stabilisation. An overview of the available literature is provided, with an emphasis on studies that try to improve our understanding of the fundamental principles from a physicochemical standpoint The second segment delves into recent improvements in nanosuspension production reductions, which will enable formulation testing during

preclinical development. Finally, the literature on subsequent nanosuspensions solidification is reviewed, with a focus on the nanocrystals' ability to dissolve quickly during additional downstream processing. [11].

V. B. Patravale et al. study nanosuspensions are a potential method of medication delivery. Since of their diverse properties and unique benefits, nanosuspensions has emerged as a viable technique for the effective delivery of the hydrophobic medicines. Commercially, techniques including media milling or high-pressure homogeneity have been utilized to make nanosuspensions. The literature has recently highlighted the engineering of nanosuspensions using emulsions or microemulsions as templates. Nanosuspensions' unique properties have allowed them to be used in a variety of dosage forms, particularly specialized delivery methods like mucoadhesive hydrogels. Nanosuspensions have been delivered by parenteral, peroral, ophthalmic, and pulmonary routes with great success. Efforts are now being made to expand their applicability in site-specific medication delivery [12].

3. METHODOLOGY

3.1.Design:

On the basis of results get from Define, Measure and Analysis phase, nanosuspension was designed and switched for verification with various evaluation parameters. The multifunctional nanosuspension was successfully created using a Six-sigma DMADV methodology, or a unique method for simultaneous estimation of AMB or DOX inside the recently developed nanosuspension was established but also validated. In term of effectiveness, precision, LOD, LOQ, or robustness, the devised approach achieves the best results. The new approach is simple and quick to use, demonstrating its suitability for regular pharmaceutical examination of these chemical components in a variety of formulations.

3.2.Sample:

All of the compounds employed in the study were of analytical quality, and the tests were conducted using double distilled water. Century Pharma Pvt. Ltd., Halol, Gujarat, India, provided pure DOX, while Akhil Healthcare Pvt. Ltd., Vadodara, Gujarat, India, provided AMB. Parul Institutes of Pharmacy, Parul University, Gujarat, Vadodara, India, supplied all of the essential high-grade chemicals.

3.3.Instruments:

For the method, Shimadzu 1800 U.V. Visible Spectrometer, Citizen analytical balance ML204/A01 (Mettler Toledo, Switzerland), ELICO pH meter LI120 (SreeBharathi Life Science, Vijayawada, India), FITR ALPHA (Bruker, Germany), Ultra sonicator (Fronline FS 4, Mumbai, India), Melting point equipment (Contech Equipment Ltd., Vashi, Mumbai, India), Magnetic stir.

3.4.Data Collections:

3.4.1. Procedure for preparation Nanosuspension using the concept of Six Sigma DMADV approach

Measure: After carefully observing the qualities of all chosen excipients, develop formulations using different excipients. Surfactant, co-surfactant, stabilizer, or organic phase combinations are all extensively examined for compatibility. The defined technique was assessed by following particular stages for nanosuspension preparation. The following are the detailed steps:

- Step 1**:** Doxofylline was mixed with Methanol to make Ambroxol HCl: varying quantities of water.
- Step 2: With a magnetic stirrer, the aforesaid mixtures were swirled for 5-10 min and well blended.
- Step 3: To establish a stable and homogenous formulation, several proportions of Tween 20/80, Zentham Gum, or PEG were explored. Then, 1 mL PEG 400 was well mixed with 0.5 mL Tween 80, 0.1 mg Zentham Gum, but also 0.5 mL Tween 80.
- Step 4: In a securely sealed container, the resultant combination was maintained at room temperatures for 20 hours and overnight.
- Step 5**:** After 24-28 hours and overnight storage, the storage precipitate was collected.

Analysis: To determine formulation compatibility, elemental analysis, FTIR, UV Spectrophotometry, zeta potential, but also dissolution tests are utilized. For the evaluation of the newly designed nano suspension, further technique development or validation was carried out.

Verify: Following the creation of new formulations, it is critical to analyze numerous parameters. The data was verified, evaluated, or analyzed, as well as the results were reported.

3.5.Data Analysis:

3.5.1. Calculation parameter of formulation:

- *Particle size data by the Zetasizer*

Particle size refers to the measures of solid particles contained in a composition. By loading a samples cell with individual nanoparticles such as using a Malvern zetasizer, the particles size of Mixture 1, Mixture 2, or Mixture 3 nanoparticles was estimated (Malvern ZS90).

- *FTIR*

Using an FT-IR spectrophotometer, the FT-IR spectra for pure drug was produced using the KBr disc technique (Bruker, Germany). The disc was made by grinding or dispersion the samples with microencapsulated IR grade KBr powder, and pressing it with a hydraulic KBr press at 7-12 kpa pressure. The disc was then analyzed using FT-IR, and the normal spectra was compared. After that, FT-IR spectra for different medications were studied or compared to reference spectra.

- *UV Spectrophotometry*

A Uv visible spectrophotometer was used to estimate the optimum wavelength of AMB but also DOX, or the findings were recorded.

- *Zeta Potential*

To increase formulation stability or shelf life while lowering formulation time and expense, zeta potential tests were performed. The zeta potential was used to evaluate the stability of the new formulation. Electrostatic but also charge repulsion magnitudes were measured or reported.

- *Dissolution study*

For 5ml of each newly designed nanosuspension formulation, in-vitro drug release was examined using a USP (type II) paddle device at a speed of 50rmp. At 37.50°C, dissolution was monitored in a 900 mL acidic media (0.1N HCl). After extracting samples for 15, 30, 45, or 60 minutes, the contents were filtered through a 0.45 membrane filter or the absorbance of the resulting solution was measured using UV Spectrophotometry at 273.5 nm after suitable dilution using U.V Spectrophotometry.

3.5.2. In a newly designed nanosuspension, one method for simultaneously determining AMB or DOX was established.

Pure drug absorptions spectra were measured between 200 and 400 nm. The scaling factor in the first derivative technique was retained at 1 as well as the delta lambda was kept at 10. The derivative ratio amplitudes were measured against increasing concentrations of pure AMB or DOX to create calibration plots. The AMB but also DOX zero crossing sites were established. For determining the wavelength of 273.5 nm in the absence of interference (Zero absorbance of the nanosuspensions and zero crossing point of the nanosuspension).

3.5.3. Analytical method for estimation of newly developed nanosuspension:

- *Solution*

Stock solution of newly created nanosuspension, 0.1 mg mL-1 in methanol:water, made separately in methanol:water with such a proportion of 0.5:9.5 for methanol or water, respectively. For additional technique verification

or application, standard solution of nanosuspension were produced and measured using a UV Visible spectrophotometer.

The suggested approach was tested for linearity, as well as range, precision, accuracy, Detection Limit (LOD), Quantification Limit (LOQ), and Robustness, in accordance with ICH criteria.

- *Linearity and Range*

A calibration curve was produced for AMB or DOX spanning concentration ranges of 2-12 g/ml or 5-30 g/ml, respectively. AMB but also DOX stock solutions with concentrations of 100 g/ml were produced separately as well as diluted for calibration curves of each medication individually. The calibration graphs for AMB and DOX were collected and published separately.

- *Accuracy*

Recovery tests were used to assess the accuracy of the suggested approach as well as interferences from excipients. The conventional addition technique was used to conduct the recovery studies (spiking method). This experiment was carried out by adding following factors of standard AMB or DOX (80 percent, 100 percent, and 120 percent of the recently developed nano suspension's labeled claim for AMB but also DOX) to a known amount of newly developed nano suspension but also calculating the percent of standard drugs recovered.

- *Precision*

The developed technique's intraday or interday accuracy were quantified in terms of percent RSD. For intraday accuracy, the trials were repeated six times a day, or for interday precision, they were performed six times on six distinct days. The percent relative standard deviation both for intraday precision or intraday accuracy was computed six times individually. Finally, the mean percent RSD was determined (percent RSD = [S/X] 100, in which S is standard deviation or X is indeed the mean of a samples studied).

- *LOD or LOQ*

According to ICH rules, the limit of detection was computed using the following equation.

LOD = 3.3 S/m, whereas S is the standard deviation of the drug's peak regions but also m denotes the slope of the calibration curve. It's stated as a 3:1 signal-to-noise ratio. According to ICH recommendations, the limit of quantification (LOQ) was computed using the following equations. LOQ = 10 S/m, where S represents the standard deviation of a drug's peak areas and m represents the calibration curve's slope. It's expressed as a signal-to-noise ratio of 10:1. On a regular basis, the LOD or LOQ values were painstakingly examined to verify that they were accurate and reproducible.

- *Robustness*

The resilience was verified by varying the flow rate or changing the wavelength slightly. The RSD % was determined, or the findings were presented.

4. RESULT AND DISCUSSION

4.1.Optimization of method for newly developed nano suspension:

As depicted in the system, different surfactants like Sodium stearate, sodium lauryl, Polysorbates 80, Polysorbates 60, Span 80, Span 20, Poloxamer 188, Poloxamer 182, Poloxamer 407; co-surfactants like Butanol, Glycerol, Sorbitol, Isopropanol, Ethanol, Potassium sorbate, PEG 400, Benzalkonium chloride, 1-butanol, Propylene glycol (PG), Caprvlic corrosive; and stabilizers like Polyvinyl pyrroidone (PVP), Carboxymethyl Cellulose (CMC), Alginate, Chitosan, Sodium lauryl sulfate (SLS), Tween 80, Tween 20, Hydroxypropyl methylcellulose (HPMC) were pursued for the advancement of nanosuspension. Different extent of solvents were pursued for improvement of Nano suspension. The best outcomes were gotten as far as definition solidness and sturdiness utilizing 0.5:9.5 methanol: water as dissolvable, 0.5ml of Tween 80 as Polysorbate surfactant, 1ml PEG 400 to keep up with the legitimate

consistency and 0.1 mg of Zentham Gum was added to get appropriate steadiness of the plan alongside thickness. The preliminaries in light of enhancement of procedure for advancement of nano suspension with results were accounted for in Table 1.

Table 1: Composition used in the Trial Batch Formulation of Nanosuspension

Sr. No.	AMB : DOX	Methanol : Water	Tween 80 (ml)	Zentham Gum (mg)	PEG 400 (ml)	Precipitate presence / absence
1	30:400	1:9	0.5	-	-	Minor
2	15:200	1:9	0.5	-	-	Absence
3	60:800	1:9	0.5	-	-	Absence
4	7.5:100	0.5:9.5	0.5	-	-	Absence
5	120:1600	0.5:9.5	0.5	-	-	Absence
6	30:400	1:9	0.5	0.1	1	Presence
7	15:200	1:9	0.5	0.1	1	Presence
8	60:800	1:9	0.5	0.1	1	Absence
9	7.5:100	0.5:9.5	0.5	0.1	1	Presence
10	120:1600	0.5:9.5	0.5	0.1	1	Absence
11	135:600	1:9	0.5	0.1	1	Absence

Except for three experimental batches of Sr. No. that according 6,7 & 9 for 30:400, 15:200, and 7.5:100 proportions of AMB: DOX correspondingly, the precipitate is not detected in all concentrations of varied proportions.

4.2.Evaluation of newly developed Nano-suspension:

Furthermore, using dissolving research, these batch of nano suspensions were tested for several evaluation criteria such as particle size, Zeta potential, IR, or drug release. The evaluation parameters' findings are provided in table 2 below:

Table 2: Result of Evaluation Parameter of Developed Formulations

Sr. No	Evaluation parameters	AMB: DOX	Optimized batch number – According to Table 1	Observation		Discussion	Conclusion
1	**Particle size**	30:400	6	PDI value	265.9	Good particle size distribution is observed with good linear distribution of the particles throughout	It can be considered.
		15:200	7		156.1		
		7.5:100	9		214.6		

						the formulation.	
2	**FTIR**	30:400	6	Frequency	1645cm^{-1}	Majorly Functional groups, C=C, C=O, O-H are observed.	The base functional groups are confirmed.
		15:200	7		1639 cm^{-1}		
		7.5:100	9		3450 cm^{-1}		
3	**Zeta Potential**	30:400	6	Zeta Potential	-1.47	Zeta potential value is observed within the limit.	On the basis of comparative study for all three batches, best results were observerd with Sr. no 9.
		15:200	7		-2.04		
		7.5:100	9		-3.06		
4	**Uv Absorption maxima**	30:400	6	λ_{max}	273 nm	The spectra of formulation is overlapped with standard with the reported wavelength as 273.5nm	This indicate the presence of both the drugs in the nanosuspension.
		15:200	7		273 nm		
		7.5:100	9		273 nm		
5	**Dissolution study**	30:400	6	f_2 value	87.02%	Release in done with specific period of time.	Best release with optimize batch number 9.
		15:200	7		84.20%		
		7.5:100	9		92.24%		

For batch no 9, the optimal solvent concentration as methanol: water was chosen in the proportion of 0.5:9.5. The batch of Sr. no. 9 of table 1 was chosen as the best batch for the newly designed nano suspension based on the formulation assessment data in table 2. For batch 9, the best results were achieved using all of the evaluation parameters. Figure 3 shows a graph of % medication release for an optimized batch.

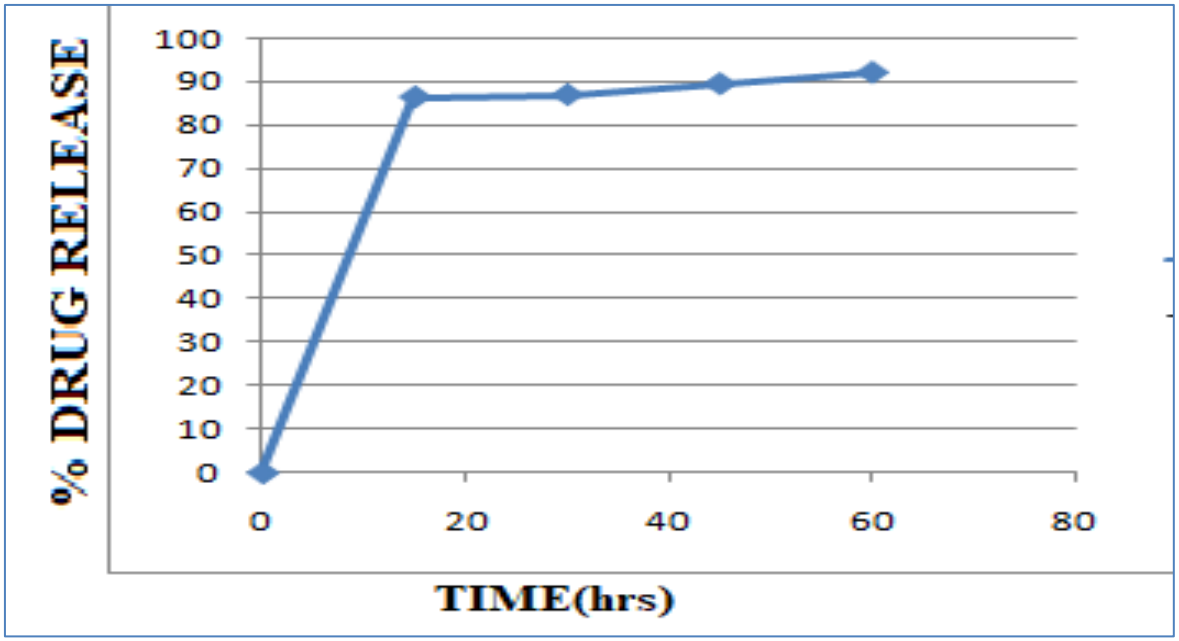

Figure 3: Drug release profile for developed Nanosuspension

A derivatives spectrophotometric technique for simultaneous determinations of AMB and DOX is also developed and validated for newly developed nano suspension.

4.3. Simultaneous determination of AMB and DOX in newly developed nano suspension

Between the concentrations of 2g/mL-1 and 12g/mL-1 and 5g/mL-1 and 30g/mL-1, AMB and DOX followed Beer's rule. Because the zero order absorption spectra of two substances, AMB and DOX, are tightly overlapped, zero order spectroscopy cannot be used to directly measure both chemicals for their determination without separation. Figure 4 depicts the zero-order spectra of pure AMB or DOX.

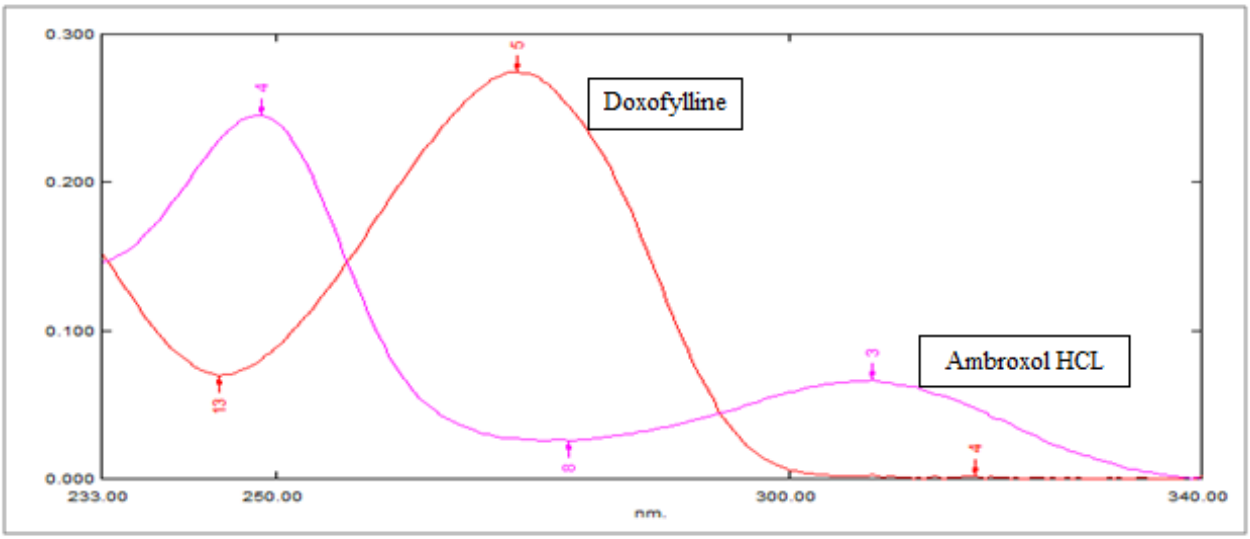

Figure 4: Zero order overlain spectra of AMB & DOX (Conc. 10µg/mL^{-1} for each)

To address this problem, the first derivatives spectra of both pure medicines were measured (as seen in the Figure 5) but also zero crossing sites for AMB or DOX were identified.

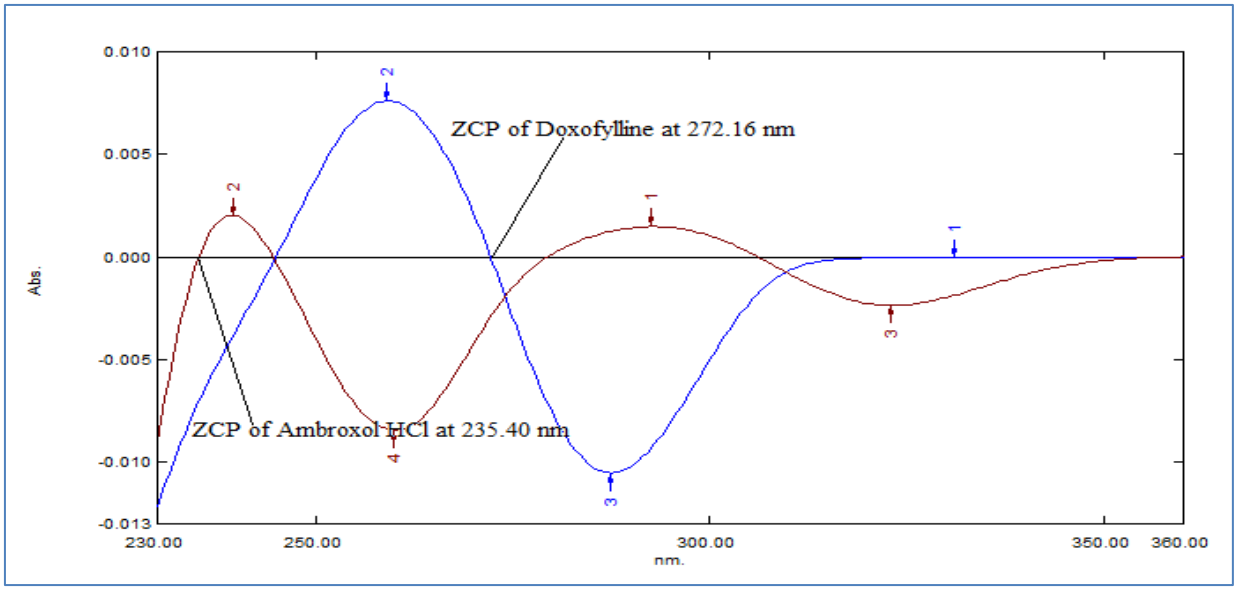

Figure 5: First derivative overlay spectra of AMB & DOX with respective zero crossing points of AMB and DOX

To construct calibration plots, the derivatives ratio amplitude were measured against increase in concentration of pure AMB or pure DOX. DOX was correctly calculated using AMB's zero crossing point, and AMB was accurately calculated using DOX's zero crossing point. As a consequence, we may compute AMB and DOX simultaneously utilizing the first order derivative zero crossing method without any prior separation or purification. The proposed approach was successfully verified for many analytical method validation parameters in accordance with ICH criteria. The standard curve for Doxofylline as well as Ambroxol hydrochloride are shown in Figures 6 and 7, respectively.

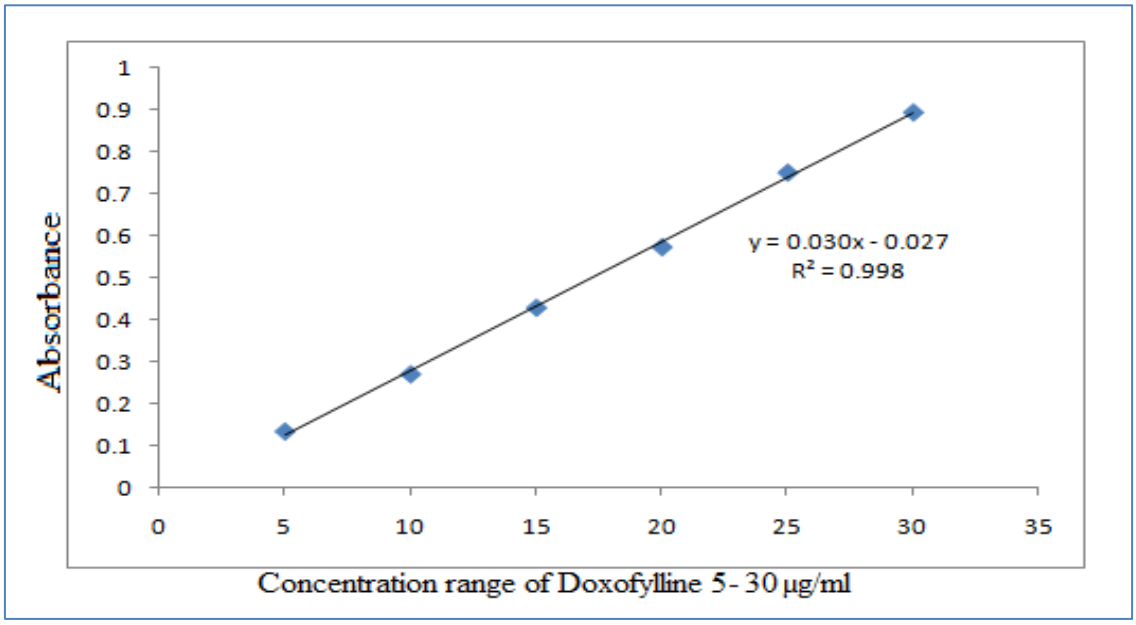

Figure 6: Illustrating the Calibration curve of Doxofylline

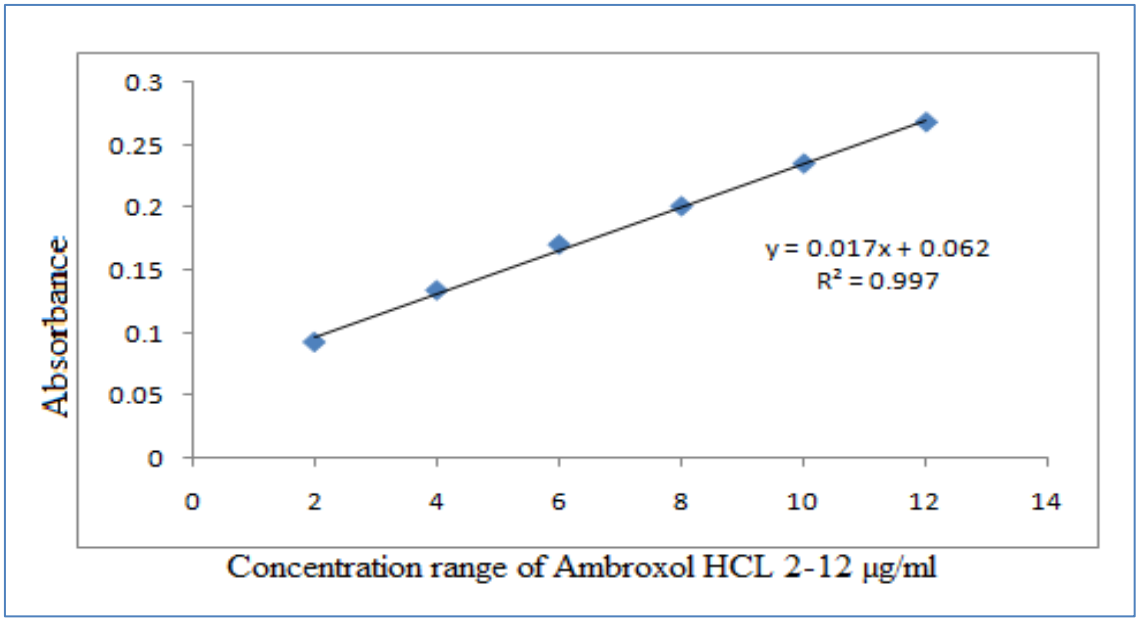

Figure 7: Illustrating the Calibration curve of Ambroxol HCl

Tables 3 and 4 show the results of accuracy study and different validation parameters, respectively.

Table 3: Results of percentage recovery of the developed method for simultaneous determination of AMB and DOX

Drug	Level of recovery	Amount of added (µg/ml)	Amount of recovered (µg/ml)	percent Amounts of recovered	Mean percent Recovery	percent RSD
AMB	80	2.4	2.39	99.58	99.58	0.87
			2.35	97.91		
			2.38	99.16		
	100	3.0	2.96	98.66	99.22	0.51
			2.98	99.33		
			2.99	93.66		
	120	3.6	3.59	99.72	99.72	0.32
			3.57	99.16		
			3.57	99.16		
DOX	80	32	31.90	99.68	99.68	0.15
			31.81	99.40		
			31.89	99.65		
	100	40	39.88	99.70	99.70	0.35
			39.60	99.00		
			39.70	99.25		
	120	52	51.86	99.73	99.73	0.09
			51.96	99.92		
			51.92	99.84		

Table 4: Validation parameters of developed UV method for concurrent determinations of AMB and DOX

Validation Parameters	AMB	DOX
Linearity range (µg/ml)	2-12 µg/ml	5-30 µg/ml

Scanning wavelength		272.16 nm	235.40 nm
Regression equations		y = 0.017x + 0.062	y = 0.030x – 0.027
Correlations coefficient (R^2)		0.997	0.998
Accuracy (Recovery study)		99.50 ± 0.2579	99.70 ± 0.0251
LOD (µg/ml)		0.353µg/ml	0.95µg/ml
LOQ (µg/ml)		1.164 µg/ml	3.135µg/ml
Precision	Interday (%RSD)	1.80	0.99
	Intraday (%RSD)	1.47	0.59
Repeatability (%RSD)		0.8 – 1.3	0.5 – 1.2
Robustness		0.27	0.19

The created approach was successfully applied to a recently produced nanosuspension, with the results shown in table 5.

Table 5: Application of the newly developed method for determination of conc. on AMB and DOX in newly developed nano suspension.

Newly developed Nano suspension		**% Amount Found [a] ± S.D.**	
AMB	DOX	AMB	DOX
2.4	32	99.58 ± 0.86	99.68 ± 0.15
3	40	95.22 ± 0.50	99.70 ± 0.35
3.6	52	99.72 ± 0.32	99.73 ± 0.096

[a] – Mean value of three determination

5. CONCLUSION

The nanosuspension for AMB or DOX was successfully produced utilizing the Six Sigma DMADV principle. With the newly produced nanosuspension, the fundamental reasoning of enhancing dissolving and oral bioavailability is effectively justified. Various assessment criteria for the newly designed nanosuspension were satisfactorily examined. Without any previous separation or purification stages, the innovative first derivative approach was effectively developed or verified for both AMB or DOX in mixed form. The new procedures were determined to be simple, specific, accurate, precise, quick, and cost-effective, indicating their suitability for regular pharmaceutical analysis. Stabilizers of several sorts were utilized to make a stable nanosuspension. As a result of the findings, it could be concluded that the unique Six-sigma DMADV technique for developing novel Nanosuspension may be employed as an effective tool for increasing solubility or oral bioavailability for medications with poor bioavailability.

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