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Development and Validation of UV-Spectrophotometric Method for Simultaneous Determination of Acamprosate calcium, Disulfiram and Ondansetron hydrochloride in bulk and tablet dosage forms.

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ABSTRACT

A mixture of acamprosate calcium, disulfiram and ondansetron hydrochloride is used for the treatment of drug abuse including alcoholism. In this study, a *UV*- spectrophotometric method was proposed for simultaneous determination of acamprosate calcium, disulfiram and ondansetron hydrochloride. Determination of these drugs was performed using the ^{1}D value of acamprosate calcium at 207 nm, ^{2}D value of disulfiram at 270 nm and ^{3D} value of ondansetron hydrochloride at 310 nm. The analysis method was validated for various parameters according to ICH guideline and linear over the range of 8-48,4-24, and 2-12 µg ml^{-1} for acamprosate calcium, disulfiram and ondansetron hydrochloride, respectively. The correlation coefficient was found to be 0.998, 0.998 and 0.999 for ACM, DIS and OND respectively. Within-day and between-day precision and accuracy values for all three compounds were within an acceptable range .The developed method was used for simultaneous determination of these drugs in pharmaceutical individual dosage forms and in laboratory mixture no interference from excipients was observed.

Keywords: Acamprosate calcium, Disulfiram, Ondansetron hydrochloride, UV-spectrophotometric method, simultaneous equation.

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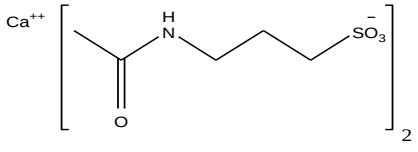
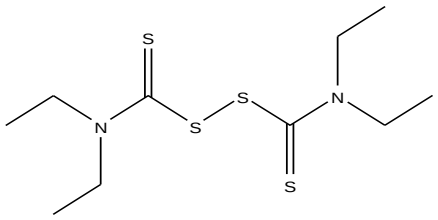
INTRODUCTION

Acamprosate calcium(ACM) chemically known as Calciumbis[3-(acetyl amino)propane-1-sulphonate], a structural analogue of γ – aminobutyric acid and an upper analogue of taurine, is a relatively new drug used to prevent relapse in weaned alcoholics[1] Dsulfiram(DIS) (bis(diethylthicarbamoyl) disulfide is a dithiocarbamate drug used clinically in the treatment of alcoholism[2.] It inhibits the enzyme aldehyde dehydrogenase that leads to the accumulation of acetaldehyde, a byproduct of alcohol metabolism, producing unpleasant and aversive side effects on alcohol consumption.It addition disulfiram, inhibits dopamine hydroxylate[3-4] Ondansetron hydrochloride(OND) is chemically (±) 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl) methyl]-4H carbazol-4-one, monohydrochloride, dehydrate and it is used as selective 5-HT3 receptor antagonist and used in the management of nausea and vomiting induced by cytotoxic chemotherapy and radio therapy and also post operative nausea, vomiting and alcohol abuse they may be used as single drug or in combination of alcohol abuse treatment[5-12]

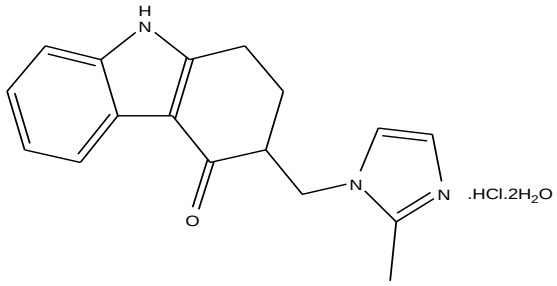
Chemical structures are shown in following Figure



Acamprosate calcium



Disulfiram



Ondransetron hydrochloride

Figure: 1, 2, 3 structures of ACM,DIS and OND respectively

A survey of literature showed that there are several spectrophotometric, HPLC or LC-MS-MS methods for determination of these drugs alone or in combination dosage forms. There has been no spectrophotometric method reported for simultaneous determination of these drugs in combination.. The development and evaluation of spectrophotometric methods can reduce the

time and cost of the analysis. Due to the spectral separation of these drugs, they could be determined by classical spectrophotometric methods. The method is very simple, rapid, and reliable techniques for simultaneous determination of multiple compounds. These methods could be used without any pretreatment procedures and tedious sample preparations. The goal of this study was to develop a practical, reliable and spectrophotometric method for the simultaneous determination of acamprosate calcium, disulfiram and ondansetron hydrochloride in a individual and multicomponent formulation.

MATERIALS AND METHOD

Chemicals:

Sodium hydroxide pellets, Potassium dihydrogen phosphate, Sodium Lauryl Sulphate, Methanol, Phosphoric acid and Distilled water.

Drugs

Acamprosate calcium were procured from Emcure Pharmaceuticals, Pune, Disufiram from Swapnaroop drugs and Pharmaceuticals Ltd. Aurangabad and Jagsonpal Pharmaceuticals Ltd., Faridabad and Ondansetron HCl was from Cipla, Kurkhumb plant, Pune, Maharashtra, India

Instrument:

Shimadzu UV-Visible spectrophotometer(model UV-1800)was employed with a spectral band width of 1 nm and a wavelength accuracy of 0.3 nm(with automatic wavelength correction with a pair of 1 cm matched quartz cell)

METHOD

Selection of solvent and wavelength:

Solubility of ACM, DIS and OND was checked in phosphate buffer pH 7.4.UV spectrum of the three drugs in this solution were recorded. The absorbance of the three was found maximum at wavelength of 207,272 and 310 nm (figure 4) were selected which are the λ max of ACM, DIS and OND respectively.

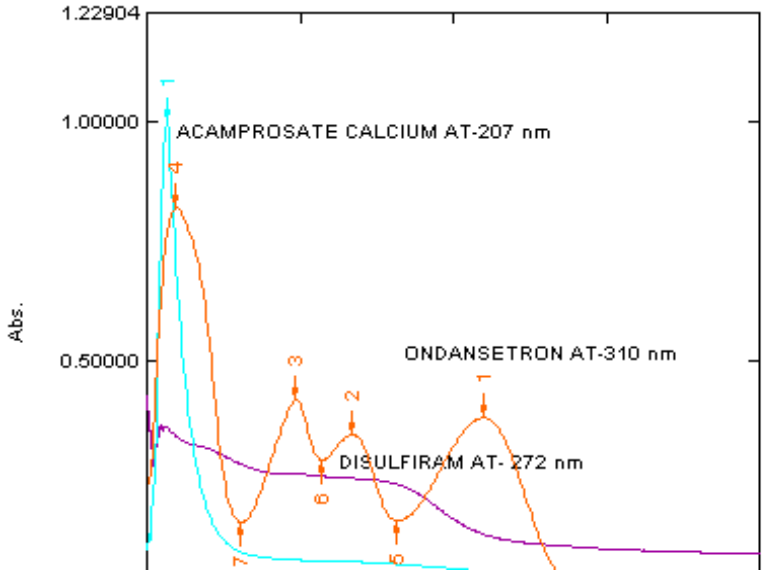


Figure 4: Overlay spectra of ACM, DIS and OND showing selected wavelength

Preparation of standard stock solutions:

ACM,DIS and OND (08:40:20mg) were separately weighed and transferred to 100 ml volumetric flask and all the three drugs were dissolved in phosphate buffer Ph 7.4 to get concentrations of 0.8,0.4 and 0.2 mg/ml respectively as a standard stock-I

Further dilutions were prepared by taking 0.1,0.2,0.3,0.4,0.5 and 0.6 ml from stock-I and diluted up to 10 ml separately to obtain the resultant concentrations of 8,16,24,32,40,48 mcg 4,8,12,16,20,24 mcg and 2,4,6,8,10,12 mcg/ml respectively for determining linearity of ACM,DIS and OND.

Application of Simultaneous equation method:

In quantitative estimation of three components by simultaneous equation method, three wavelengths i.e.,207 nm of ACM,272 nm of DIS and 310 nm of OND were selected as their respective λ max from the overlain spectrum, at which three drugs have maximum absorbance. The concentrations of three drugs in the mixture can be calculated using the following equations[8].

$$C\ ACM = \frac{(\mathrm{A1(ay2az3 - az2ay3) - ay1(A2az3 - az2A3) + az1(A2ay - ay2A3)})}{\mathrm{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}}$$

$$C\ DIS = \frac{(\mathrm{ax1(A2az3 - az2A3) - A1(ax2az3 - az2ax3) + az1(ax2A3 - A2ax3)})}{\mathrm{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}}$$

$$C\ OND = \frac{(\mathrm{ax1(ay2A3 - A2ay3) - ay1(ax2A3 - A2ax3) + A1(ax2ay3 - ay2ax3)})}{\mathrm{ax1(ay2az3 - az2ay3) - ay1(ax2az3 - az2ax3) + az1(ax2ay3 - ay2ax3)}}$$

Where C ACM,C DIS and C OND are the concentrations of ACM,DIS and OND respectively in mixture and sample solutions.A1,A2 and A3 are the absorbances of sample at 207,272 and 310 nm respectively,ax1,ax2 and ax3 are the absorptivity of ACM at 207,272 and 310 nm respectively,ay1,ay2 and ay3 are the absorptivity of DIS at 207,272 and 310 nm respectively, ,az1,az2 and az3 are the absorptivity of OND at 207,272 and 310 nm respectively.

Analysis of individual marketed formulations:

For the analysis mixture of composition were prepared and from individual marketed tablets formulation containing ACM, DIS and OND 20 tablets were weighed and their average weight was determined. The tablets were then crushed to fine powder and powder equivalent to weight of one tablet in a ratio of 4:2:1 was transferred to 100 ml volumetric flask and dissolved in 50 ml of phosphate buffer pH 7.4 and shaken for 5 minutes in sonicator. Finally the volume was made up to the mark with same solution and then filtered through Whatmann filter paper. From this solution,1ml was pipette out in to a 10 ml volumetric flask and diluted up to the mark with phosphate buffer pH 7.4.From above solution 0.2 ml was transferred in to a 10 ml volumetric flask and diluted with phosphate buffer pH 7.4 up to the mark. The absorbance of above solution of both was measured at 207,272 and 310 nm. The concentration of each analyte was determined using the simultaneous equation.

RESULTS AND DISCUSSION

The analytical method was validated with respect to parameters such as linearity, precision, limit of detection(LOD),limit of quantitation (LOQ) and accuracy.

Linearity:

Calibration graph was found to be linear that is adherence to the system of Beer's law which was found over the concentration range of 8-48,4-24, and 2-12 µg ml-^{1} for ACM,DIS and OND respectively(Figure5-7).Absorbance and concentration was subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients. The regression data as given in table 1, showed a good linear relationship.

Table 1: Linearity

Parameter	ACM	DIS	OND
Linearity range	8-48 µg ml-1	4-24 µg ml-^{1}	2-12 µg ml-^{1}
Correlation coefficient	0.998	0.998	0.999
Slope	0.035	0.046	0.035
Intercept	0.043	0.010	0.007

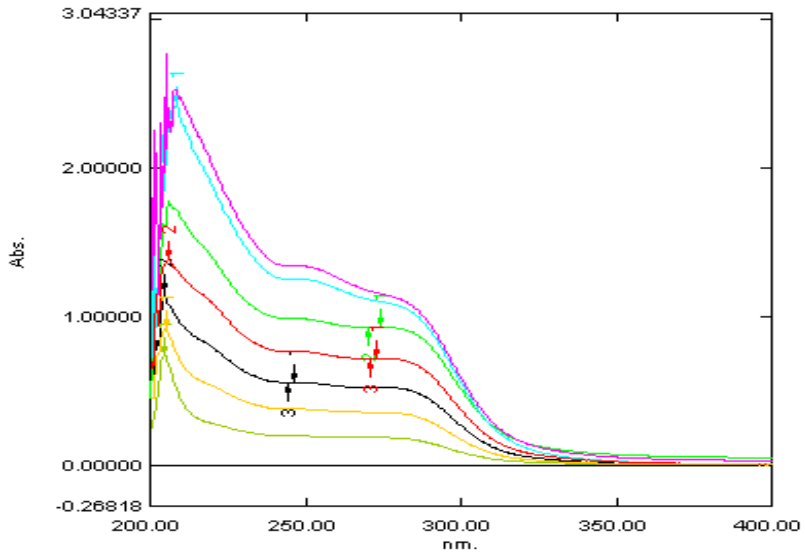


Figure 5.Overlay spectra of Standard Acamprosate calcium (8-48 µg ml-^{1})

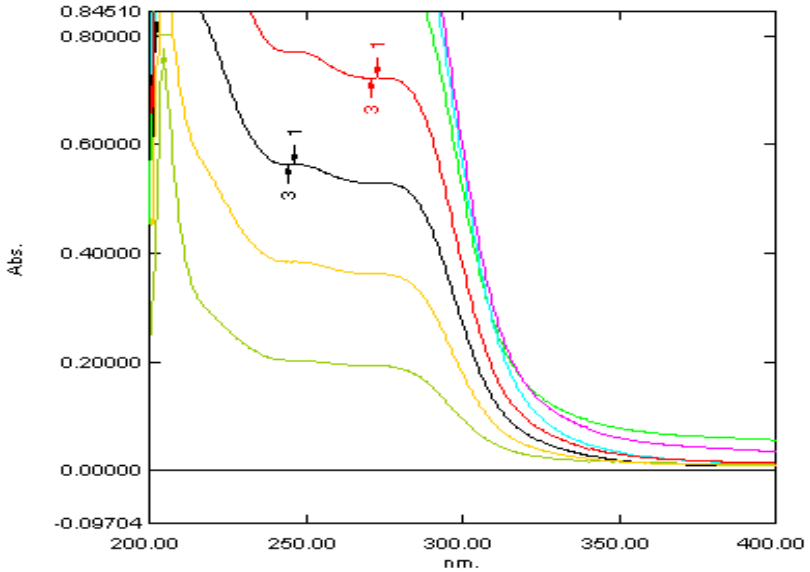


Figure 6.Overlay spectra of Standard Disulfiram (4-24 µg ml-^{1})

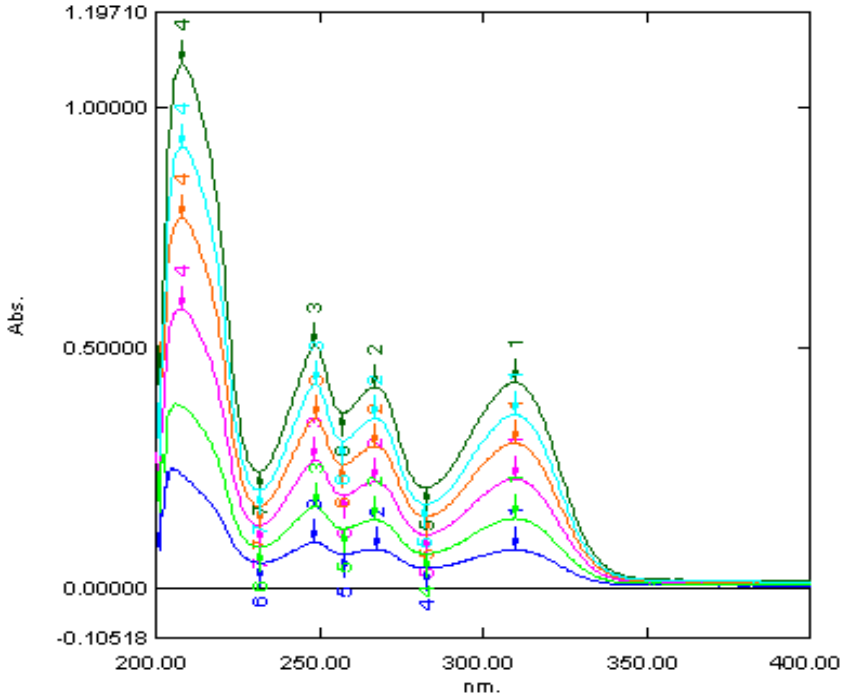


Figure 7.Overlay spectra of Standard Ondansetron hydrochloride (2-12 µg ml-^{1})

Precision:

To check the degree of repeatability of the method, suitable statistical evaluation was carried out. The concentrations of three drugs were measured five times on the same day at intervals of 1 Hr and on five different days for intra and inter day study, respectively. The Relative Standard Deviation (% RSD) was found to be less than 2.The results were shown in table 2.

Table 2: Precision studies

Drug	Concentration (µg ml-^{1})	Intraday precision %*RSD	Interday precision %*RSD
ACM	24	0.5295	0.3023
DIS	12	0.4285	0.3545
OND	6	0.901	1.1819

*mean of five observations

LOD and LOQ:

LOD and LOQ was found to be 0.095 µg ml^{-1} and 0.2858 µg ml-^{1} for ACM, 0.032µg ml-^{1} and 0.0967 µg ml-^{1} for DIS, 0.056 µg ml-^{1} and 0.1697 µg ml-^{1} for OND respectively.

Accuracy:

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by the standard addition method. The recovery studies were carried out at three different levels i.e.80%, 100% and 120% level. The percentage recovery values were shown in table 3

Table 3: Accuracy

Drug	% Recovery			% *Relative Standard Deviation		
Level	80%	100%	120%	80%	100%	120%
ACM	100.32	100.54	99.46	0.0003	0.1613	0.238
DIS	99.96	99.18	100.05	0.241	0.4439	0.4301
OND	99.92	99.87	99.88	0.0713	0.1925	0.0583

*mean of five observations

Table 3: Analysis of formulation

Drug	Labeled amount (mg/tablet)	Amount estimated (mg/tablet)	% Label claim	%*RSD
ACM	333	332.97	99.99	
DIS	500	499.43	99.89	
OND	8	7.9983	100.03	

*mean of five observations

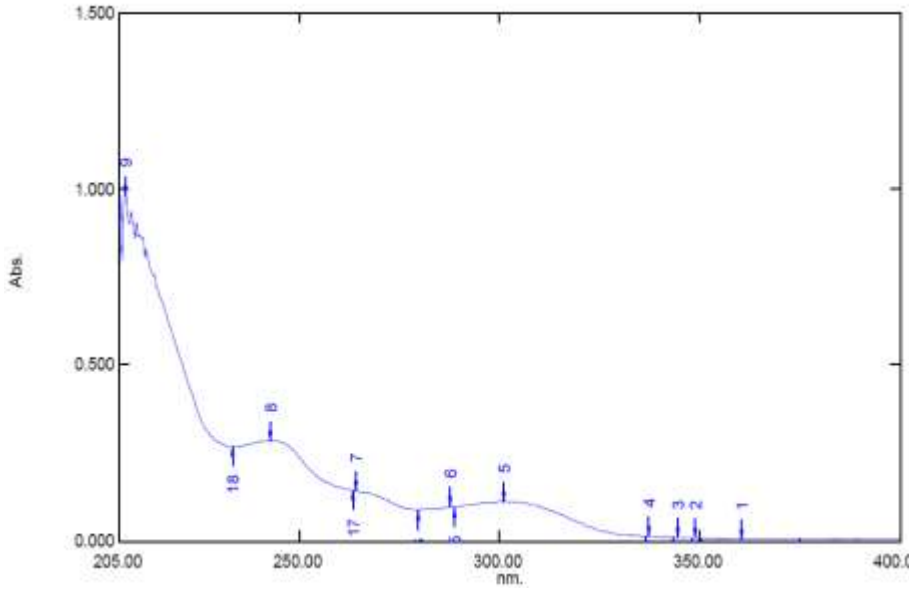


Figure 8.Overlay spectra of formulation mixture containing ACM, DIS and OND.

CONCLUSION:

The developed UV spectrophotometric method is simple, precise, accurate, linear, reproducible and repeatable for the estimation of ACM, DIS and OND in pharmaceutical dosage forms without any interference from the excipients. It can be successfully applied for the routine analysis of all the three drugs in physical mixture and pharmaceutical dosage forms including novel drug delivery formulations like implantable tablets etc.

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