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Estimation of amlodipine and valsartan from micro tablets

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Abstract
Oral route of drug administration is the most convenient method of administering drugs. Among the oral route of drug administration solid oral dosage forms represent the most preferred dosage form. Oral solid dosage forms facilitate accurate dosing of drugs compared to oral liquid dosage forms. Patient's compliance is better due to the convenience of self administration of drugs. Oral dosage form is broadly classified into two categories: Single-unit and Multiple-unit dosage forms.
The basic concept of multiple-unit systems is that the dose of active ingredient is released by the individual subunits and the functionality of the entire dose depends on the quality of the subunits. Micro tablets are tablets with a diameter to or smaller than 2 to 3 mm. The main advantage of micro tablets over other dosage forms is less chances of gastric irritation and dose dumping. It has advantage over pellets in more accuracy in drug content and ease of manufacturing. The present study is aimed at developing micro tablets with 2mm diameter of combinational product of Amlodipine and Valsartan. The current research is aimed at developing an estimation method for quantification of Amlodipine and Valsartan from micro tablets in a capsule.

Keywords: micro tablets, multiple unit dosage forms, estimation, amlodipine, valsartan

1. Introduction
Micro tablets used as multiple unit dosage forms exhibit several advantages over the single unit dosage forms. Micro tablets have lesser risk of dose dumping. It exhibits Minimal local irritation in the Gastro intestinal tract.
The micro tablets can be filled into hard gelatin capsules for individual customized dosing. Because of the definite shape, uniform size and surface, and high acceptable strength, micro-tablets can maintain their structure and shape in a more reproducible way than usual pellets or granules, during the manufacturing as well as post manufacturing. The micro tablets manufacturing are easier than pellets. The manufacturing involve conventional compression machine.
The only additional requirement is multi tip tooling. The micro tablets can be effectively combined to develop fixed dose combination products and also the incompatible drugs can be combined in capsules during capsule filling, based on the therapeutic requirement to treat concurrent diseases. Micro-tablets can be coated with functional as well as non functional film coating due to their constant specific surface area compared to granules, smooth outer surface and robust mechanical properties to alter the release profile of the dosage form.
Depends upon the dose requirement the number of micro tablets can be increased or decreased for filling into the capsule. Dose titration is easily possible for especially the treatments like hypertension where long term therapy is required and to be adjusted as per the therapy. The preparation of micro-tablets is of rising importance in pediatrics due to new European regulatory requirements on products for pediatric use (Regulation (EC) No. 1901/2006, 2006).
First clinical studies revealed that even children of small age are able to swallow mini-tablets of 3.0 mm diameter (Thomson *et al*., 2009). Therefore, rational of development of micro-tablets is of high impact with particular interest in robust mechanical properties and high drug loads. A combination drug most commonly refers to a fixed dose combination (FDCSs), which is a formulation including two or more active pharmaceutical ingredients (APIs) combined in a single dosage form.

2. Material and Methods
Valsartan and Amlodipine Besylate were obtained as a gift sample from Lupin Ltd, Mumbai. Microcrystalline Cellulose (Avicel PH 102, FMC Bio Polymer), Croscarmellose Sodium (AcDiSol, FMC Bio Polymer), Sodium lauryl Sulphate (Kolliphor, BASF), Colloidal Silicone Dioxide (Aerosil 200 Evonik), Magnesium Stearate (Hyqual 2257 vegetable source, Avantor).

2.1 Amlodipine micro tablets and valsartan micro tablets in Capsule
Various formulations were prepared based on the formula mentioned in Table 1A for Amlodipine Micro Tablet and Table 1B for Valsartan micro tablet.
Microcrystalline Cellulose is used as the diluents in both Amlodipine and Valsartan Micro tablet. Croscarmellose sodium is used as the disintegrant in both the micro tablet. Colloidal Silicone dioxide is used to improve the flow ability of the blend in both the blend. Being a direct mixing and compression manufacturing process flow ability is very important to ensure the content uniformity and weight variation. Sodium Lauryl Sulphate is used as the surfactant to improve the solubility of Valsartan in Valsartan micro tablet.

Table 1A: composition of Amlodipine Micro tablet

Composition	**Mg/Micro Tab**
Amlodipine Besylate	3.465
Croscarmellose Sodium	0.400
Microcrystalline Cellulose	3.855
Colloidal Silicone Dioxide	0.200
Magnesium Sterate	0.080
Total	8.000

Table 1B: composition of Valsartan Micro tablet

Composition	**Mg/Micro Tab**
Valsartan	4.00
Microcrystalline Cellulose	2.72
Croscarmellose Sodium	0.80
Sodium Lauryl Sulphate	0.20
Colloidal Silicone Dioxide	0.20
Magnesium Sterate	0.08
Total	8.00

Table 1C: Each size #0 hard gelatine capsule contains micro tablets of Amlodipine and Valsartan as per below table

Product Name	**Aml/Valsartan 5/160**		**Aml/Valsartan 10/160**	
Active	**Amlodipine**	**Valsartan**	**Amlodipine**	**Valsartan**
Strength	5	160	10	160
No of Micro tablets	2	40	4	40
Total Micro Tablets	42		44	

2.2 Evaluation of Micro Tablets

A. Weight variation

20 Micro tablets were weighed individually in a Mettler Toledo analytical balance and the mean and weight variation was checked. The weight variation was within 10% limit.

B. Hardness

The hardness of the mini-tablets produced was determined in a TBH ERWEKA® hardness tester using 10 micro-tablets of each formulation

C. Friability

Dust-free sample (6.5g) of micro-tablets was accurately weighed and analyzed in an ELECTROLAB® Friability Tester. None of the tablets were broken in both the formulation.

3. Sample Preparation

Stock methanolic solution of amlodipine and valsartan of concentration 100 µg/ml and 200 µg/ml respectively were separately prepared by dissolving the appropriate amount of the respective substance in methanol. Working standard solutions used for method optimization were prepared freshly before application by mixing suitable aliquots of stock solutions of amlodipine and valsartan and diluted with methanol to obtain the required concentration ranges.

Stock methanolic solution of laboratory prepared mixture of amlodipine and valsartan containing 100 µg/ml and 1.6 mg/ml respectively was prepared by dissolving 25 mg amlodipine and 400 mg valsartan in 250 ml methanol. Tablets working solution was prepared by dissolving an accurately weighed quantity of powdered micro tablets sample containing an equivalent of 10 mg amlodipine and 160 mg valsartan was transferred into 100 ml volumetric flask, then 70 ml methanol was added. The mixture was sonicated for ten minutes and the volume was made up using methanol, then the sample was filtered.

4. Estimation procedure of amlodipine and valsartan

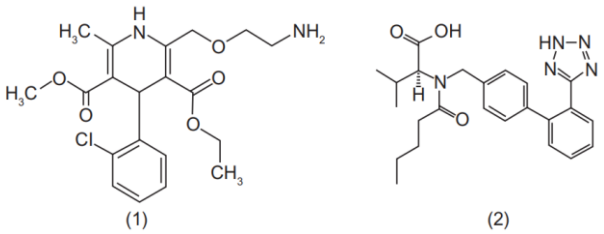

Fig 1: Amlodipine (1) and valsartan (2) structure formula.

Aliquots (1–8 ml) or (1–9 ml) of amlodipine or valsartan stock solutions respectively were transferred into two separate series of 10 ml volumetric flasks and volumes were made up with methanol. The absorbance spectrum of each flask were recorded at zero order between 200–450 nm and stored in a personal computer.

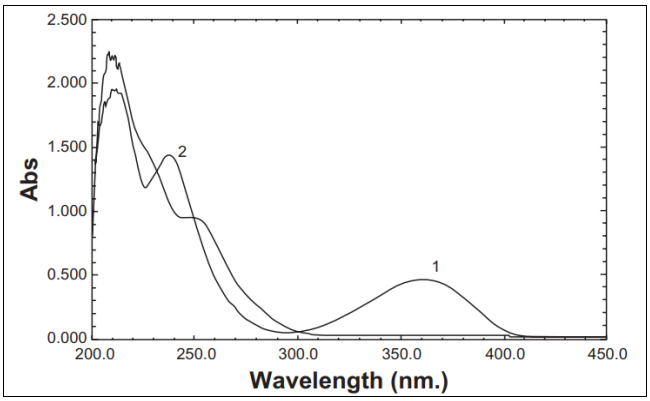

Fig 2: UV spectrophotometric spectrum of methanolic solution of (1) amlodipine (45 µg/ml) and (2) valsartan (60 µg/ml).

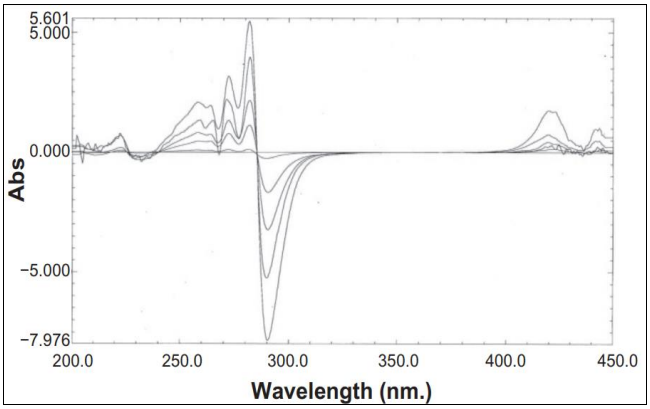

Fig 3: First derivative of ratio spectra of different concentrations of valsartan using spectrum of amlodipine (40 µg/ml) as a divisor spectrum.

Table 1: Analytical parameters from calibration graph in quantification of amlodipine and valsartan by the proposed method.

Regression Data	Amlodipine	Valsartan
Linearity	10-80 µg/ml	20-180 µg/ml
Slope	0.011	0.086
Intercept	0.0003	0.101
r (Correlation Coefficient)	0.9996	0.9997
r^2 (Correlation of determination)	0.999	0.999
LOD	5.5 µg/ml	8 µg/ml
LOQ	8.5 µg/ml	12 µg/ml

The absorbance of amlodipine series was determined in zero order at 360.5 nm. The UV absorption spectra of the series of valsartan solutions were divided wavelength by wavelength by a standard spectrum of amlodipine (40 µg/ml). The first derivative of the obtained ratio spectra with $\Delta\lambda = 4$ nm and scaling factor equal 10 were used for determination of valsartan concentration from the amplitude at 290 nm.

5. Determination of laboratory prepared mixture

The method was applied for the determination of laboratory prepared mixture of amlodipine and valsartan containing 100 µg/ml and 1.6 mg/ml respectively. Aliquots (1–8 ml) and (1.5–10 ml) of the stock methanolic solution of laboratory prepared mixture were transferred into two separate series of 10 ml and 100 ml volumetric flasks, volumes were made up using methanol. The first series of (10 ml) volumetric flasks was used for the determination of amlodipine, while the other series (100 ml volumetric flasks) was used for the determination of valsartan applying the same mentioned procedure.

6. Determination of amlodipine or valsartan from micro tablets

Aliquots of 1.5, 4 and 8 ml of tablets working solution were transferred into two separate series of 10 ml and 100 ml volumetric flasks for determination of amlodipine and valsartan respectively.

To study the accuracy of the proposed method and check the interference from excipients present in the dosage form, recovery experiment was carried out by standard addition method. This study was performed by addition of different amounts of amlodipine and valsartan to a known concentration of the commercial tablets.

Table 2: Results of quantitative analysis of amlodipine and valsartan in bulk powder by the proposed method.

Taken		Found		% Recovery	
Aml*	Val**	Aml	Val	Aml	Val
10	20	10.09	20.01	100.90	100.55
25	40	24.88	40.12	99.5	100.30
45	80	45.16	79.32	100.35	99.15
65	140	65.59	139.52	100.90	99.66
75	180	74.48	179.84	99.30	99.88
M±SD				100.19±0.76	99.91±0.55

Notes : *Aml= Amlodipine, **Val = Valsartan

Table 3: Results of quantitative analysis of amlodipine and valsartan in laboratory prepared mixture by the proposed method.

Taken concentration of each drug in mixture		Found		% Recovery	
Aml*	Val**	Aml	Val	Aml	Val
15	24	14.90	23.89	99.33	99.55
20	32	20.22	32.27	101.10	100.84
40	64	39.94	64.64	99.85	101.00
60	96	60.53	95.22	100.88	99.19
80	128	79.55	129.16	99.44	100.90
M±SD				100.12±0.820	100.30±0.857

Notes : *Aml= Amlodipine, **Val = Valsartan

Table 4: Results of quantitative analysis of amlodipine and valsartan in laboratory prepared mixture by the proposed method three times within the same day (repeatability).

Taken		Found						Standard Deviation	
		*Aml			**Val				
Aml	Val	1st	2nd	3rd	1st	2nd	3rd	Aml	Val
30	48	30.8	30.42	29.17	47.66	48.36	48.36	0.85	0.62
50	80	50.25	49.98	49.38	80.15	80.62	79.13	0.45	0.76
65	104	63.97	65.54	64.76	104.90	103.98	103.74	0.78	0.61
80	128	80.14	79.85	80.62	127.92	128.56	128.60	0.39	0.38

Notes : *Aml= Amlodipine, **Val = Valsartan

Table 5: Results of quantitative analysis of amlodipine and valsartan in laboratory prepared mixture by the proposed method over three different days (reproducibility).

Taken		Found						Standard Deviation	
		*Aml			**Val				
Aml	Val	1st	2nd	3rd	1st	2nd	3rd	Aml	Val
30	48	29.57	30.43	29	49.31	47.86	48.52	0.72	0.73
50	80	49.66	49.13	50.51	80.39	81.45	79.72	0.67	0.87
65	104	65.55	66.05	64.74	105.37	103.87	104.68	0.66	0.75
80	128	79.69	79.22	80.85	129.10	127.98	128.66	0.84	0.56

Notes : *Aml= Amlodipine, **Val = Valsartan

Table 6: Results of quantitative analysis of amlodipine and valsartan in micro tablets by the proposed method.

Taken		Found		% Recovery	
Aml*	Val**	Aml	Val	Aml	Val
15	24	14.95	24.36	99.66	101.5
40	64	39.58	63.57	98.95	99.33
80	128	80.88	129.1	101.10	100.86
M±SD				99.9±1.1	100.56±1.1

Notes : *Aml= Amlodipine, **Val = Valsartan

Table 7: Results for the application of the standard addition technique for the determination of amlodipine and valsartan in micro tablets by the proposed method.

Taken from tablets (µg/ml)		Found of tablets		Added standard (µg/ml)		Found of added standard (µg/ml)		% Recovery of added standard	
*Aml	**Val	Aml	Val	Aml	Val	Aml	Val	Aml	Val
15	24	14.95	24.36	25	40	25.21	39.58	100.85	98.94
				45	72	45.45	72.56	101.00	100.74
				104	104	64.72	103.82	99.57	99.83
40	64	39.58	63.57	15	24	15.08	24.13	100.53	100.54
				20	32	20.08	32.36	100.38	101.12
				35	56	34.74	55.81	99.26	99.66
M±SD								100.27±0.70	100.15±0.81

Notes : *Aml= Amlodipine, **Val = Valsartan

7. Result and Discussion

The UV spectra of amlodipine and valsartan in zero order show that amlodipine has a significant peak at 360.5 nm at which the spectrum of valsartan does not interfere (Fig. 2). So methanolic solution of amlodipine was quantitatively determined at 360.5 nm, Beer's law was obeyed over concentration range 10–80 µg/ml. The amlodipine concentration could be calculated using the following equation.

$$A=0.011C+0.003$$

Valsartan spectrum in zero order shows a complete overlapping with that of amlodipine (Fig. 2). Applying first, second and third order spectra did not resolve valsartan peaks. So first derivative of the ratio spectra was applied to determine valsartan in presence of amlodipine. The influence of $\Delta\lambda$ and the effect of the divisor concentration on the calibration graph for the proposed mixture was studied in order to select the best factor for the determination. Results indicate that $\Delta\lambda = 4$ was the most suitable one, while divisor concentration range 10–60 µg/ml gave good results, 40 µg/ml was used overall. For the determination of valsartan the absorption spectra of valsartan was divided by that of standard amlodipine solution (40 µg/ml). The first derivative of the developed ratio spectra were calculated with $\Delta\lambda = 4$ and scaling factor equal 10. (Fig. 3) shows that valsartan could be determined by measuring the amplitude at 290 nm. The proposed method is applicable over the concentration range 20–180 µg/ml. Valsartan concentrations could be determined applying the following equation.

$$A = 0.086C + 0.101$$

Parameters of regression equations of both drugs are collected in Table 1.

Accuracy of the method was tested and results show M ± SD of 100.19 ± 0.76 and 99.91 ± 0.55 for amlodipine and valsartan respectively, (Table 2). In order to demonstrate the validity and applicability of the proposed method, recovery studies were performed by analysing laboratory prepared mixture of amlodipine and valsartan with different composition ratio (Table 3). Results of the tested samples within day (repeatability) and between days (reproducibility) show standard deviation between results less than one indicating high degree of precision (Tables 4 and 5). The proposed method can be conveniently used to determine both amlodipine and valsartan in micro tablets (Table 6). The validity of the method was assessed by applying the standard addition technique for the determination of amlodipine and valsartan in micro tablets; satisfactory results results were obtained (Table 7).

8. Conclusion

In this research a new spectrophotometric method was developed to identify amlodipine and valsartan in mixture without previous separation. The method was optimized and validated. The method was successfully applied for the determination of amlodipine and valsartan in micro tablets.

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