

Assessment of Endogenous Biochemical Composites Emphasizing Drug Interaction of Cardiovascular Combined Dosage Formulation in Marketed Product

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Abstract

The study reveals the investigational approaches of a Cardiovascular formulated tablet dosage i.e, Atorvastatin (ATVS) & Olmesartan (OLM) after getting negative feedbacks on pharmaceutical market.

A simple, sensitive, precise and rapid analysis under highly sophisticated LCMS/MS system to evaluate the endogenous biochemical composites like Aldosterone (ALD), Angiotensin-II (ANG-II) and Mevalonate (MVA) in plasma concentration level, which associate with mentioned drugs pharmacology. The methods were developed and validated for the same to analyze plasma concentration level among 20 patient volunteers pre-administered with targeted combined tablet dosage.

Chromatographic peaks of standard and internal standard exhibits excellent regression curve line and correlative coefficient, $r^2 = 0.998, 0.999$ & 0.999 of ALD, ANG-II & MVA respectively. The quality control profile of accuracy, mean% recovery is range between 90.6-99.13% & 88.2-96.3% respectively of endogenous bio-analytes. And inter-day & intraday precision % RSD (Relative Std. Dev.) is ranges from 1.60-1.90 of the same. Analytical reports represent the lower concentration of ALD after ATVS (Atorvastatin)+OLM (Olmesartan) therapy compare to without drug. But, in case of ANG-II, it is completely inverse and MVA conc. lowers equally in ATVS+OLM and ATVS (individual therapy).

ALD & ANG-II are physiologically responsible for hypertension while MVA for cholesterol biosynthesis. Thus, study concludes that OLM bioavailability approaches get retarded in compare to unchanged bio-availability of ATVS in the combined (ATVS+OLM) formulation and stands weak antihypertensive activity compare to individual therapy, reason might be due to pharmacokinetic interaction. So, its fails the expected synergism.

Keywords: Pharmacovigilance study; Endogenous biochemical composites; Cardiovascular formulation; Drug interaction; LCMS/MS analysis

Introduction

One of the Pharmaceutical tablet dosage form which categorized under Cardiovascular combined formulation (Atorvastatin 10 mg & Olmesartan 20 mg) discloses Clinical, Marketed, patients and community health feedbacks and reports high morbidity rate on chronic stage therapy. Although logics behind Atorvastatin (ATVS) & Olmesartan (OLM) combination i.e, Anti-atherosclerotic & antihypertensive must flourish best antihypertensive activity, but in real it's just reverse. The false propitious prescription with this formulation is unsafe for health and thus required post marketed re-investigation as per concerning Pharmacovigilance.

A very simple, sensitive, precise and innovative way to detect the reason of drug-interaction is to analyze endogenous biochemicals like Aldosterone (ALD), Angiotensin-II (ANG-II) and Mevalonate (MVA) conc. level on systemic circulation through LCMS/MS, which is directly variants these drugs therapy.

Many historic instrumental system involved in evaluation of ALD level [1-15]. But recently LCMS/MS is the most promising sensitive, simple and accuracy detective system [16].

Similarly, ANG-II is the endogenous polypeptide detected still now with column switching techniques [17-19] and HPLC cum radioimmunoassay [20-23]. This ALD and ANG-II are the biochemical composites tools on detection of Olmesartan effectiveness in dosage formulation.

And, Cholesterol biosynthesis precursor MVA detection is

responsible to study the Atorvastatin activity role in combined dosage formulation treatment. The most sophisticated determination of MVA is through LCMS/MS [24].

Methodology

Study design

The study on human volunteers was carried under approval of ethics committee "HURIP Independent Bioethics", Ibrahimpur Road, Kolkata, India. The study was performed along with 20 patient volunteers consent and under supervision of Doctor. The collection of blood plasma was basically done three times per individual for 7-14 days- in first stage without any drug administration. Secondly, along with combination of OLM+ATVS and thirdly with only single hypertensive drug i.e, Olmesartan. First stage was for 1 week in which the patients were devoid of any antihypertensive drugs. The patients were evaluated for demography and measurement of BP followed by withdrawal of blood to quantify the levels of ALD, ANG-II & MVA.

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Sample extraction procedure

The thawed frozen ALD sample added with 500 ml methanol/L of water and then liquid-liquid extraction was done with dichloromethane and ethyl-ether in ratio 60:40. Then vortex, centrifuged, upper layer of organic phase separated, dried and reconstituted with 350 µl of methanol/water.

The plasma sample of ANG-II was added with 500 µl of 0.5% formic acid, loaded to pre-conditioned cartridge and finally eluted with 5 ml methanol containing 5% formic acid. It was dried and reconstituted to 1 ml of 16% Acetonitrile in 0.1% formic acid in water before loading to LCMS/MS.

The plasma sample containing MVA of 500 µl were added to a glass tubes contain IS (100 µl, 200 ng/ml), 0.1 N HCl (1 ml), and water (0.5 ml), vortex mixed, kept 30 min aside and transferred to a solid-phase extraction preconditioned cartridge. Washed with 0.1 N HCl (1 ml) followed by water (1 ml) and 15% methanol in water. Dried in N2 stream and residue were reconstituted in 0.2% ammonium hydroxide solution (100 µl) to convert MVAL to MVA, before injection to LCMS/MS.

Standardization and validation of biochemical composites-ALD, ANG-II & MVA

Standardization of ALD, ANG-II & MVA was prepared with conc.

ranges 50-800 ng/mL, 5-200 ng/mL and 50-1000ng/mL respectively, with standard composite powders ALD, ANG-II & MVA and with corresponding Internal Standard -ALD (D-7), ANG-III & MVA (D-7). The validation profiles including- Specificity & selectivity, linearity, precision & accuracy, Extraction recovery, LOD & LOQ, Stability for the LCMS/MS detection system implicate to evaluate biochemical composites conc. level in plasma.

Result and Discussion

The plasma spiked ALD std vs. Aldosterone-d7 (Internal Standard; IS) calibrated curve represents regression line linearity equation of $y=2.7917+1.001x$, coefficient of regression, $r^2 =0.998$. Method performance was evaluated through quality control -Low Quality Control (LQC), Middle Quality Control (MQC) & High Quality Control (HQC) for accuracy, 96.4 %, 97.9 % and 98.73%; for mean % Recovery- 91.2%, 93.4% & 89.7% and for inter-day & intraday precision - 1.79, 1.90 & 1.75 and 1.90, 1.63 &1.79 respectively as %RSD. The method was confirmed for sensitivity by estimating Limit of Detection (LOD)-0.13 ng/ml and limit of Quantification (LOQ)-0.432 ng/ml.

The plasma spiked ANG-II (std) and ANG-III (IS) calibrated curve represents regression line linearity equation of $y= 0.985x + 0.861$ and excellent correlation coefficient of regression $r^2= 0.999$ [25]. Method performance was evaluated through quality control-LQC, MQC &

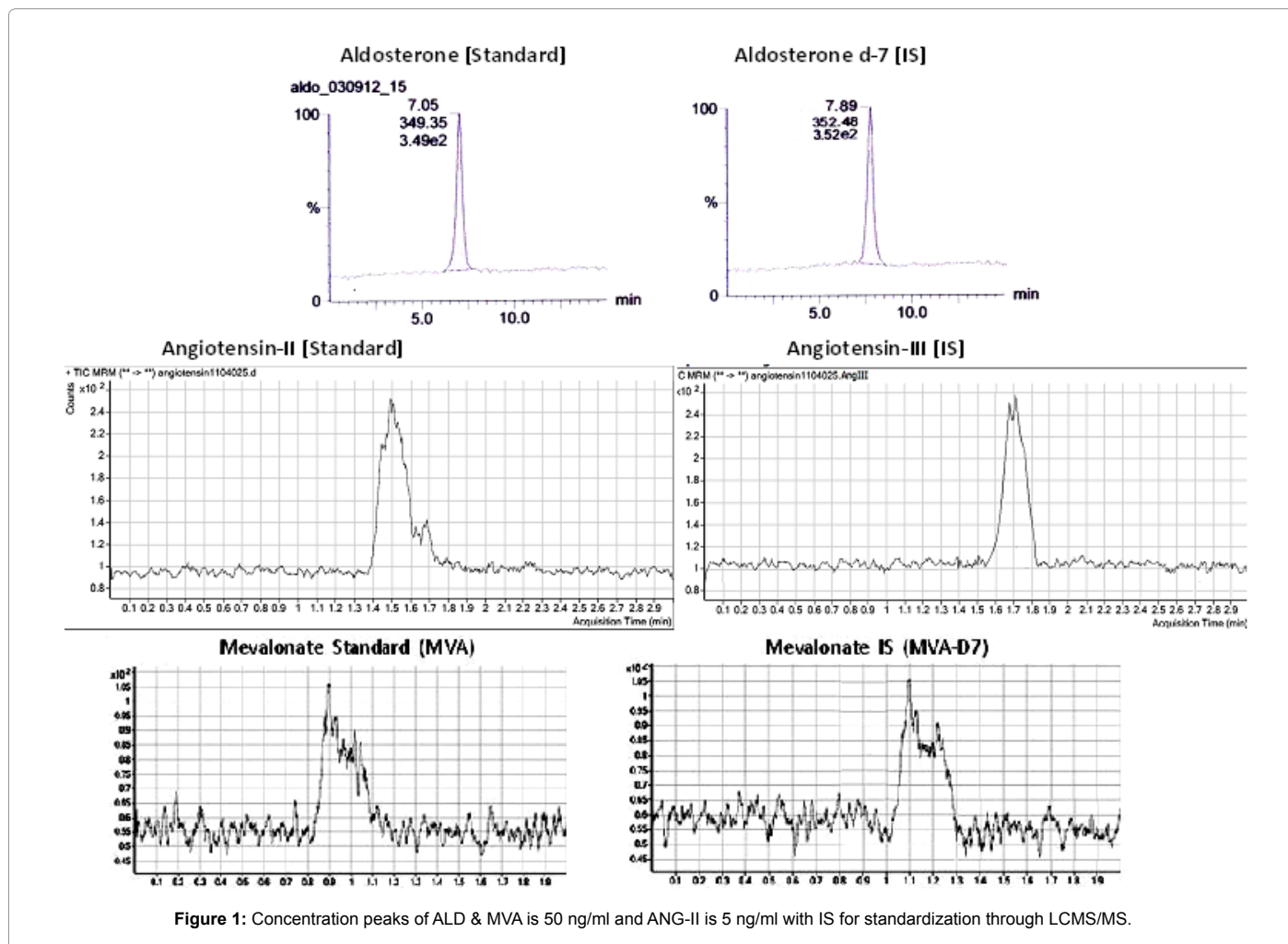


Figure 1: Concentration peaks of ALD & MVA is 50 ng/ml and ANG-II is 5 ng/ml with IS for standardization through LCMS/MS.

Sr. No.	Endogenous Bio-chemical composite detected in plasma	Mean Plasma concentration of ALD, ANG-II & MVA of Twenty patients in three different stages of treatment			
		Without Drugs Conc. in ng/mL	ATVS + OLM Conc. in ng/mL	OLM Conc. in ng/mL	ATVS Conc. in ng/mL
1	ALD	60.3325 ± 1.33	12.024 ± 0.4606	ND	—
2	ANG-II	4.4917 ± 0.06519	10.5715 ± 0.06820	17.61363 ± 0.108	—
3	MVA	74.90054 ± 0.6646	49.58125 ± 0.65797	—	50.91677 ± 0.484

Table 1: Mean Plasma concentration of endogenous biochemical composites detected in human plasma in three different stages.

Sr. No.	Patients Gender	Age	Body Weight	Body Height	Blood pressure (Systolic & Diastolic pressure) measurement & blood collection (4 ml) in three stages		
					Without drug	ATVS + OLM	OLM
					124/82 (Control)	122/82 (Control)	120/79 (Control)
1	Male	28	84	5.8ft	176/117	169/113	160/110
2	Female	29	65	5.3 ft	182/ 123	175/120	164/112
3	Male	23	83	5.7 ft	185/126	180/119	168/102
4	Male	27	86	5.9 ft	165/119	162/115	156/104
5	Male	27	79	5.6 ft	171/128	167/122	161/111
6	Male	26	81	5.6 ft	157/110	150/106	143/100
7	Female	32	82	5.8 ft	179/119	170/110	145/89
8	Male	29	77	5.7 ft	184/130	173/124	152/112
9	Male	26	73	5.6 ft	174/121	170/115	147/92
10	Male	28	85	5.10 ft	169/118	166/114	156/100
11	Female	28	66	5.2 ft	172/122	165/112	142/88
12	Female	30	69	5.5 ft	177/125	168/117	137/113
13	Male	33	65	5.4 ft	172/119	166/110	149/104
14	Male	28	66	5.3 ft	186/123	169/114	151/102
15	Male	29	72	5.8 ft	182/131	166/119	146/90
16	Male	32	69	5.2 ft	163/116	161/110	151/89
17	Male	32	80	5.9 ft	179/131	170/125	166/112
18	Female	33	65	5.1 ft	183/134	175/131	162/107
19	Male	30	69	5.5 ft	179/122	176/114	156/85
20	Female	26	62	4.9 ft	165/125	159/120	150/100
Mean ± SEM			78.27 ± 2.21	5.57 ± 0.0721	175 ± 1.81 /122.95 ± 1.33	167.85 ± 1.50 /116.50 ± 1.35	153.10 ± 1.91** /101.12 ± 2.09**

Table 2: The effect of different antihypertensive treatment over the Blood pressure (Systolic/Diastolic) of Hypertensive Human volunteers. Values are Mean ± SEM (n=20), **P<0.0001 when compared to without drug treated condition.

HQC for accuracy, 90.6 %, 94.86% and 97.13%; for mean % Recovery- 93.1%, 91.7% & 88.2% and for inter-day & intraday precision - 1.86, 1.62 & 1.68 and 1.60, 1.73 & 1.90 respectively as %RSD. The method was confirmed for sensitivity by estimating LOD (0.16 ng/ml) and LOQ(0.52 ng/ml).

The plasma spiked MVA (std) and MVA-D7 (IS) calibrated curve represents regression line linearity equation of $y=1.0205x-10.0154$ and excellent correlation coefficient of regression $r^2=0.999$. Method performance was evaluated through quality control – LQC, MQC & HQC for accuracy, 95.0%, 95.67% and 99.13%; for mean % Recovery- 96.3%, 92.7% & 87.8% and for inter-day & intraday precision - 1.82, 1.77 & 1.69 and 1.85, 1.68 & 1.72 respectively as % RSD. The method was confirmed for sensitivity by estimating LOD (0.97 ng/ml) and LOQ (2.33 ng/ml).

The Standardization of the method developed after tracing through LCMS/MS is on Figure 1 representation at their least concentration i.e, 50, 5, 50 ng/ml for ALD, ANG-II & MVA respectively.

The concentration of ALD is comparatively high in patients without drug than ATVS+OLM and not detectible in OLM due to very low concentration level. But, the conc. of ANG-II is just reverse to ALD as these both are inversely proportional to each other (Table 1) according to detection time. Thus, comparatively lower tendency of lowering ALD

concentration after combined therapy retains its high BP in arterial vessel. And reversely, low conc. of ANG-II in plasma after combination therapy is due to high competitive affinity of ANG-II for the ANG-receptor-1 against low conc. of OLM (ANG-receptor-1 blocker) in blood, which disposes less extra unbounded ANG-II in blood and which is vice-versa to individual OLM therapy. In the case of MVA, the conc. of ATVS & ATVS+OLM is nearly equal but declined to without drug, i.e, unaffected to BP (Table 2).

Conclusion

The endogenous biochemical composites-ALD & ANG-II concentration level in plasma is only responsible for indicating low conc. bioavailability of OLM in blood on combined dosage compare to individual OLM. Thus, its concludes that quantitative analysis of endogenous biochemical composites -ALD & ANG-II emphasizes the in-vivo assessment of drug-interaction between the chemically stable ATVS & OLM in a tablet dosage formulation.

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