

## SINGLE RP-HPLC METHOD FOR THE QUANTIFICATION OF CANDESARTAN AND HYDROCHLOROTHIAZIDE IN FORMULATIONS

### ABSTRACT

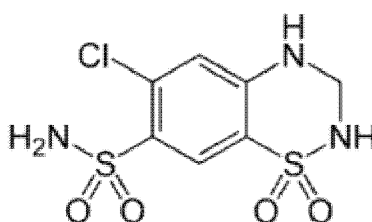
**Objectives:** To develop a single RP-HPLC method for determination of candesartan and hydrochlorothiazide in formulation. **Methods:** Chromatographic separation was achieved on a C18, 250 x4.6mm, 5 $\mu$  column. Mobile phase composed of methanol, acetonitrile and 0.1% Ortho phosphoric acid in the ratio of 35:50:15v/v with 1.0ml per min flow rate and detection was at 272 nm. Injection volume is 10 $\mu$ L and runtime was selected for 12min. **Results:** High resolution was achieved and retention time of hydrochlorothiazide and Candesartan are about 4.58min and 6.94min, respectively. The absorbance of both active peaks were a linear function of concentration in the range 30-90 for both hydrochlorothiazide and Candesartan. The correlation co-efficient value also within the limit (0.999). **Conclusions:** Proposed single HPLC method was validated with specificity, linearity, accuracy, reproducibility and ruggedness. The method has applicable for regular analysis.

**Keywords:** Candesartan, Hydrochlorothiazide, Formulations and RP-HPLC method.

### Introduction:

#### Hydrochlorothiazide:

Hydrochlorothiazide is a thiazide diuretic (water pill) used to treat fluid retention (edema) in people with congestive heart failure, cirrhosis of the liver, or kidney disorders, or edema caused by taking steroids or estrogen. This medication is also used to treat high blood pressure (hypertension). It is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones.<sup>[1]</sup> and also used for hypercalciuria, Dent's disease and Ménière's disease. Thiazides are also used in the treatment of osteoporosis. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney, and by directly stimulating osteoblast differentiation and bone mineral formation.<sup>[2]</sup>



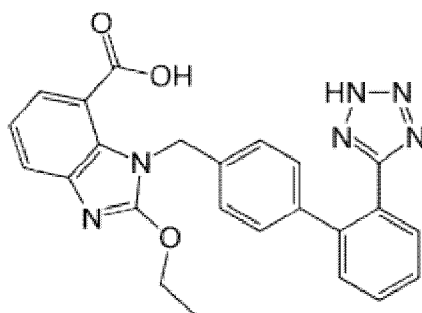
**Fig:1 Structure of Hydrochlorothiazide**

Hydrochlorothiazide belongs to the thiazide class of diuretics that acts by inhibiting the kidneys' ability to retain water. It reduces blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na<sup>+</sup>-Cl<sup>-</sup> co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport.<sup>[3]</sup> Additionally, by other mechanisms, HCTZ is believed to lower peripheral vascular resistance.<sup>[4,5]</sup>

Hypokalemia, an occasional side effect caused by Hydrochlorothiazide. Other side effects include Hypomagnesemia, Hyponatremia, Hyperuricemia and gout, High blood sugar, Hyperlipidemia, Hypercalcemia, Headache, Nausea/vomiting, Photosensitivity, Weight Gain, Gout, Pancreatitis.

### Candesartan:

Candesartan is an angiotensin II receptor antagonist used alone or in combination with other drugs to treat high blood pressure. It also is used for reducing the chance of death or hospitalization due to heart failure. It was approved by the FDA in 1998.



**Fig:2 Structure of Candesartan**

It is in a class of drugs called angiotensin receptor blockers (ARBs) which includes losartan (Cozaar), valsartan (Diovan), and irbesartan (Avapro). Angiotensin, formed in the blood by the action of angiotensin converting enzyme (ACE), is a powerful chemical that attaches to angiotensin receptors found in many tissues but primarily on smooth muscle cells surrounding blood vessels. Angiotensin's attachment to the receptors causes the muscle cells to contract and the blood vessels to narrow (vasoconstrict) which leads to an increase in blood pressure. Candesartan blocks the angiotensin receptor and thereby prevents the action of angiotensin. As a result blood vessels expand and blood pressure is reduced. Results from the CHARM study in the early 2000s demonstrated the morbidity and mortality reduction benefits of candesartan therapy in congestive heart failure.<sup>[6]</sup> Thus, while ACE inhibitors are still considered first-line therapy in heart failure, candesartan can be used in combination with an ACE to achieve improved mortality and morbidity.

The most common side effects of candesartan include headache, dizziness, fatigue, abdominal discomfort, diarrhea, and upper respiratory infections, hyperkalemia, impotence.

## MATERIALS AND METHODS

### Chemicals and reagents

Reagents used like Acetonitrile, Methanol, Water which are of HPLC grade were purchased from E. Merck, Mumbai, India. Ortho phosphoric acid is Lr-grade purchased from local store.

### Instrumentation and analytical conditions

The analysis of the drug was carried out on Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, variable wave length programmable UV/visible detector SPD-10AVP and rheodyne injector (7725i) with 20µl fixed loop. Chromatographic analysis was performed using Inertsil ODS C-18 column with 250 x 4.6mm internal diameter and 5µm particle size. Shimadzu electronic balance (AX-200) was used for weighing. Isocratic elution with MeOH 35%, ACN 50%, (0.1%) OPA 15% was selected with a flow

rate of 1.0 ml min<sup>-1</sup>. The detection wavelength was set at 272 nm with a runtime of 10 min. The mobile phase was prepared freshly and it was degassed by sonicating for 5 min before use. The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature.

#### **Preparation of Stock, working standard solutions and Sample solutions**

Standard solutions of concentration 1000 ppm of Hydrochlorothiazide and 1000 ppm of Candesartan were prepared separately using methanol. From standard solutions further solution are prepared by using methanol.

The formulation tablets of Hydrochlorothiazide (Hydrochlorothiazide -25mg) and Candesartan (Atacand -32mg) were crushed to give finely powdered material. Powder equivalent to 10 mg of drug was taken separately in 10 ml of volumetric flask containing 5 ml of Methanol and was shaken to dissolve the drug and then filtered through Ultipor N<sub>66</sub> Nylon 6,6 membrane sample filter paper. Volume of the filtrate was adjusted to the mark with the same solvent then further diluted to obtain concentration of 1000 ppm. 1ml from the each of the two drug solutions were taken and mixed well and injected into HPLC system and the resultant assay was calculated based on the peak areas.

#### **Optimization of HPLC Method**

All drugs were subjected to chromatographic analysis using mobile phases of differing pH, flow rate using the under mentioned chromatographic conditions. The changes in the retention time of all drugs were noted as a function of changing mobile phase, pH, flow rate, strength and selectivity. Finally methanol: Acetonitrile: OP in the ratio of (35: 50:15) at flow rate of 1.0 mL/min was finalized which gave acceptable retention time, plates and good resolution for Hydrochlorothiazide and Candesartan(Figure:3)

#### **Validation of the method**

Validation of the optimized HPLC method was carried out with respect to the following parameters.

**Linearity and range:** The mixed standard solution (1000 ppm of Hydrochlorothiazide and 1000 ppm of Candesartan) was further diluted to get Hydrochlorothiazide and Candesartan concentration in the range of 30-90 ppm. Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

**Precision:** The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of concentration 80ppm for Hydrochlorothiazide and 50ppmL for Candesartan six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

**Limit of detection and limit of quantitation:** Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Hydrochlorothiazide and Candesartan was made from the standard stock solution. The samples were injected in LC system and measured signal from the samples was compared with those of blank samples.

**Robustness of the method:** To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of mobile phase and wavelength. Robustness of the method was done at three different concentration level 50 ppm for Hydrochlorothiazide and Candesartan.

**Specificity:** The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. The peak purity of Hydrochlorothiazide and Candesartan was determined by comparing the spectrum at

three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E). Effect of excipients of formulation was studied for whether it interfered with the assay.

**Accuracy:** Accuracy of the method was carried out by applying the method to drug sample to which know amount of Hydrochlorothiazide and Candesartan standard powder corresponding to 25, 50 and 75 % of label claim had been added (Standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

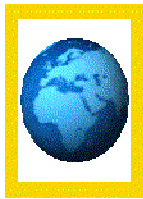
**Analysis of a marketed formulation:** To determine the content of Hydrochlorothiazide and Candesartan in tablets twenty tablets were weighed; their mean weight determined and finely powdered separately. The weight of the tablet triturate equivalent to 10 mg of Hydrochlorothiazide and 10 mg Candesartan was transferred into a 10 mL volumetric flask containing 5 mL methanol, sonicated for 30 min and diluted upto 10 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solutions 50 ppm for Hydrochlorothiazide and Candesartan separately. 1ml from the each of the two drug solutions were taken and mixed well and injected into HPLC system. The peak areas were measured at 272 nm and concentrations in the samples were determined.

## RESULTS AND DISCUSSION

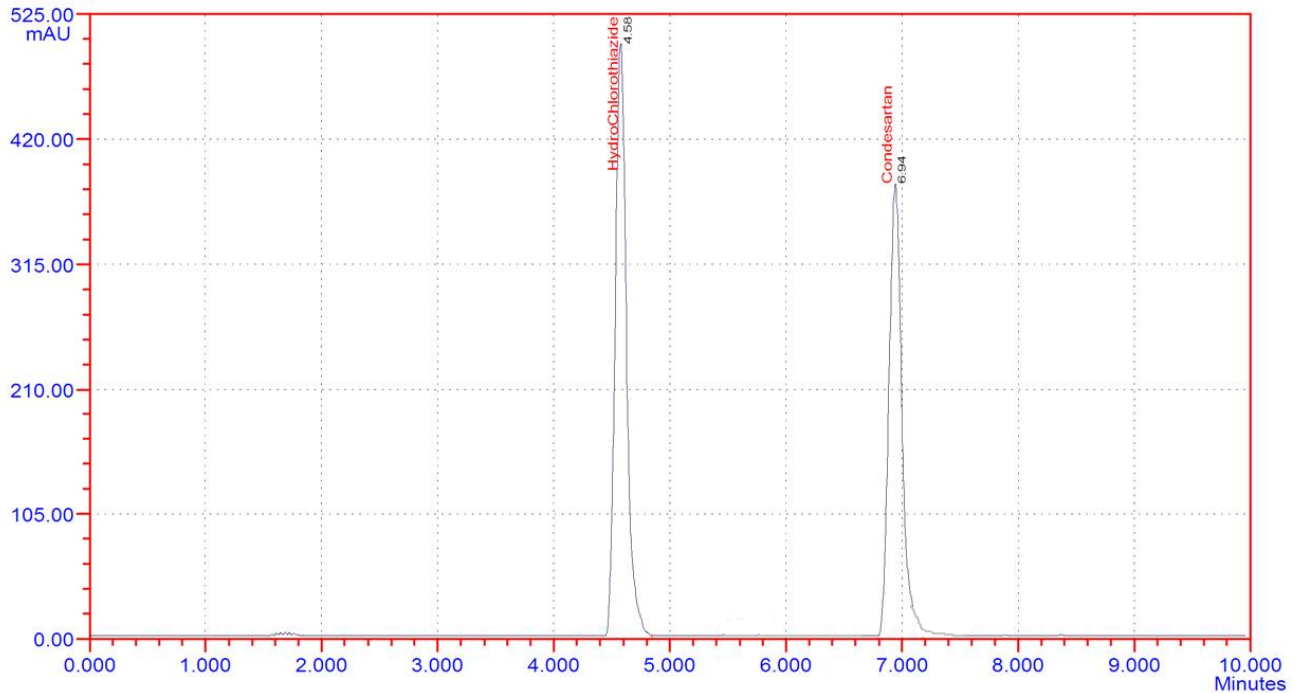
The results of validation studies on simultaneous estimation method developed for Hydrochlorothiazide and Candesartan in the current study involving methanol: CAN : OP (35: 50:15) are given below.

H.P.L.C CONDITIONS		Isocratic
ELUTION		25 micro gram/ml
A.P.I	CONC	MEOH 35%, ACN 50%, (0.1 % )OPA 15 %
MOBILE PHASE		4.6
PH		C18, 250 ×4.6 mm
COLUMN		272 nm
WAVE LENGTH		1 ml/min
FLOW		12 Min
RUNTIME		Isocratic
RETENSION TIME	Hydrochlorothiazide	4.58 min
	Candesartan	6.94 min
AREA	Hydrochlorothiazide	347270
	Candesartan	300653
TH.PLATES	Hydrochlorothiazide	8703
	Candesartan	15623
TAILING FACTOR	Hydrochlorothiazide	1.50
	Candesartan	1.29
PUMP PRESURE		10.5mpa

Table 1 : Standard Chromatogram of Hydrochlorothiazide and Candesartan



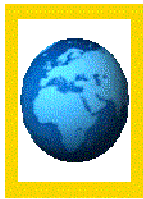
### HPLC Report



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plata
1	HydroChlorothiazide	4.578	50126	347270.3	53.597	1.50	8703
2	Candesartan	6.943	38338	300653.3	46.403	1.29	15623
Sum:			88464	647923.6	100.0000		

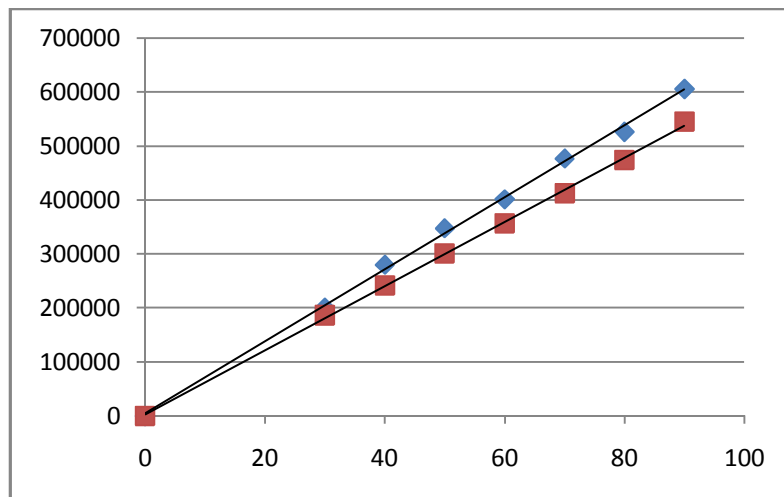
**Fig: 3 Standard Chromatogram**

**Linearity:** Hydrochlorothiazide and Candesartan showed good correlation coefficient ( $r^2 = 0.999$  for Hydrochlorothiazide and  $0.999$  for Candesartan) in given concentration range (30-90 ppm for both Hydrochlorothiazide and Candesartan). The values of the slope and intercept were 6661.9 and 4833.2 for Hydrochlorothiazide and 5942.7 and 2410.9 for Candesartan respectively.



S.No	Concentration	Area	
		Hydrochlorothiazide	Candesartan
1	30	200235	186396
2	40	280034	241628
3	50	347270	300653
4	60	401203	356319
5	70	476731	412399
6	80	525889	473313
		0.999	0.999
<b>Result</b>	Correlation Coefficient	4833	2411
	Intercept	6662	5943
	Slope		

Table 2: Linearity results



Graph:1 Linearity

**Precision:** The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines 13-15.

S.No	Concentration	Area	
		Hydrochlorothiazide	Candesartan
1	50ppm	349475	301804
2		349448	305045
3		351890	300414
4		348945	307014
5		349545	301931
6		348704	303045
Result	RSD	0.33	0.8

**Table 3: Intraday Precision results**

S.No	Concentration	Area	
		Hydrochlorothiazide	Candesartan
1	50ppm	348807	299525
2		346045	298838
3		351138	304613
4		348419	302788
5		352707	300918
6		347403	302418
Result	RSD	0.7	0.72

**Table 4 : Interday Precision results**

**LOD and LOQ:** Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 0.3ppm and 10ppm for Hydrochlorothiazide and 0.5 ppm and 1.5 ppm for Candesartan, respectively.

**Robustness of the method:** Each factor selected (except columns from different manufacturers) was changed at three levels (-0.1, 0 and 0.1). One factor at the time was changed to estimate the effect. Thus, Robustness was performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas were observed (Table 4).

Parameter	Change	Area of Hydrochlorothiazide	Area of Candesartan	% Change of Hydrochlorothiazide	% Change of Candesartan
Standard Mobile Phase	.....	347270	300653	.....	.....
	MEOH 33%, ACN 52%, OP 15 %	342784	302541	1.29	0.63
	MEOH 37%, ACN 48%, OP 15 %	349547	301609	0.66	0.32
Wavelength	273nm	345245	301184	0.58	0.18
	271nm	343448	297193	1.10	1.15
Flow Rate	1.1ml/min	352829	299233	1.60	0.47
	0.9 ml/min	342885	298529	1.26	0.71

**Table 5: Robustness results**

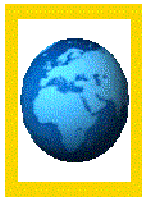
**Recovery:** As shown from the data in Table 3 good recoveries of the Hydrochlorothiazide and Candesartan in the range from 98.15 to 101.30 % were obtained at various added concentrations.

% of Recovery	Concentration in ppm	Hydrochlorothiazide		Candesartan	
		Concentration Obtained	% of Assay	Concentration Obtained	% of Assay
25%	50	49.84	99.68	49.78	99.56
	50	49.14	98.28	49.33	98.67
50%	50	50.32	100.65	50.44	100.84
	60	60.68	101.13	60.66	101.17
	60	60.51	100.84	60.39	100.65
100%	60	59.26	98.77	59.50	99.17
	80	78.52	98.15	79.06	98.82
	80	80.40	100.50	80.49	100.62
	80	80.59	100.74	81.04	101.30

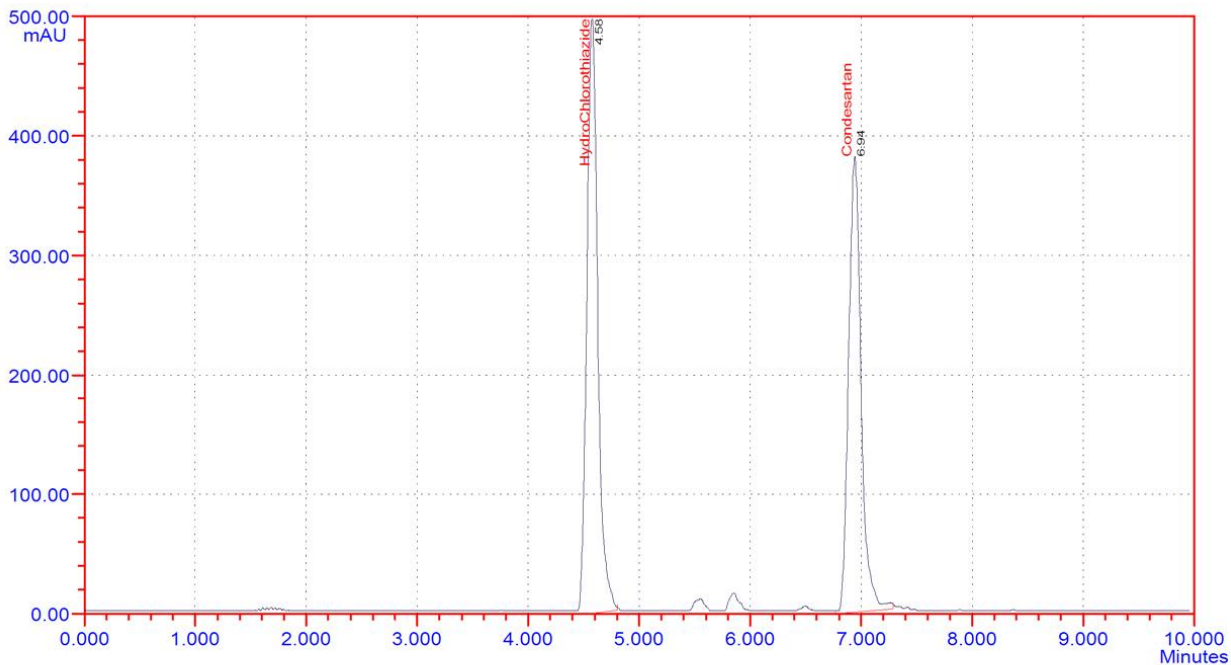
Table 6: Recovery results

**Analysis of a formulation:** Experimental results of the amount of Hydrochlorothiazide and Candesartan in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. The drug content was found to be 98.34% for Hydrochlorothiazide and 99.19 % for Candesartan. Two different lots of Hydrochlorothiazide and Candesartan tablets were analyzed using the proposed procedures as shown in Table 6.





### HPLC Report



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Hydrochlorothiazide	4.578	49954	341502.8	53.384	1.40	8938
2	Candesartan	6.943	38125	298204.4	46.616	1.75	15705
Sum:			88079	639707.2	100.0000		

**Fig: 4 Formulation Chromatogram**

Drug	Formulation	Dosage	Concentration	Amount found	% Assay
<b>Hydrochlorothiazide</b>	Hydrochlorothiazide	25mg	50 ppm	49.17	98.34
<b>Candesartan</b>	ATACAND	32mg	50 ppm	49.59	99.19

**Table 7: Formulation results**

## CONCLUSION

HPLC method was developed and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds. The drug was analysed by HPLC method using Chromosil C18, 250 ×4.6 mm with isocratic conditions and simple mobile phase containing MEOH 35%, ACN 50%, OP 15 % at flow rate of 1.0 mL/min using UV detection at 272 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 30-90 ppm for both Hydrochlorothiazide and Candesartan. The LOD and LOQ were found to be 0.3ppm and 10ppm for Hydrochlorothiazide and 0.5 ppm and 1.5 ppm for Candesartan, respectively. Statistical analysis proves that the method is suitable for the analysis of Hydrochlorothiazide and Candesartan as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Hydrochlorothiazide and Candesartan and also for its estimation in plasma and other biological fluids.

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