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Determination of Sodium (2-(2, 6-dichloroanilino) phenyl) Acetic Acid in Human Plasma by Rapid and Sensitive HPLC Method and UV-Spectrophotometry: Its Comparative Evaluation

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Authors' contributions

This work was carried out in collaboration between all authors. Author SH has supervised the designed research work and evaluated the results critically. Author JC conducted the research activities in collaboration with co-authors and wrote the first draft of the manuscript. Author KB evaluated the results and guided about protocols of thesis writing and help out in manuscript writing. Author HY and AM gathered the literature review data and arranged the data and references according to guidelines of thesis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The objectives of current study were to develop and validate a simple, rapid, and sensitive HPLC method for determination of sodium (2-(2, 6- dichloroanilino) phenyl) acetic acid (SDPAA) in human plasma according to US-FDA guidelines and determination of SDPAA by UV-Spectrophotometry.
Methodology: In case of HPLC method**,** the mobile phase composed a mixture of acetonitrile (ACN) and phosphate buffer (pH 6.8) in a ratio of 40:60 ml (pH 3.5). A Shimadzu HPLC machine (HPLC 10 ATVP) with a column Chromolith-R high resolution RP–18 end caped and a column

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length of 100mm to 4.6 mm, with a UV detector was used. The peak was observed at wavelength of 281 nm. The sample was injected at a flow rate of 1.5 ml per min. The solvent run time was 8 minutes and the average retention time of the six sample observed was 5.66 minutes. In case of UV-spectrophotometric method, Shimadzu UV-1800 spectrophotometer was used. Mixture of phosphate buffer of pH 6.8:ACN was selected as a solvent for determination of SDPAA at 281 nm. Beer law was obeyed in the range of 2-22 µg/ml. The results of both UV-spectrophotometric and HPLC methods in determination SDPAA, were compared.

Results: SDPAA was detected at 281nm and eluted at 5.669 min (without plasma) while the samples extracted from the plasma eluted at 5.667 min. The average % RSD was less than 2%. Accuracy was confirmed with the recovery studies and by three test assays. Accuracy was tested at three %age level that is within 95–99%. The linear range of 0.5-20 µg/ml (for HPLC) showed the regression coefficient (R^2) 0.999 and linear range of 2-22 µg/ml (for UV method) showed the regression coefficient (R^2) 0.998 which were in acceptable range.

Conclusion: The developed HPLC method was simple, rapid, reliable, specific and sensitive with ability to determine drug concentrations from human plasma.

Keywords: SDPAA; HPLC method; human plasma and UV-spectrophotometry.

1. INTRODUCTION

SDPPA belongs to the class of non-steroidal anti-inflammatory drugs (NSAID) or cyclo-oxygenase (COX) inhibitors. It is very effective anti-inflammatory, analgesic and antipyretic drug. It is mostly used in the curement of acute and chronic analgesia, rheumatoid and osteoarthritis [1]. Chemically, it is 2-(2,6- dichlorophenyl) amino benzeneacetic acid 4-(3H-1,2,dithiol-3-thione-5-yl)phenyl ester and is a low molecular weight agent (MWt: 318.13) [2].

The new uses under consideration of SDPAA are as bacteriostatic in UTI caused by *E.Coli*, and as anti-uricosuric agent in Alzheimer's disease [3-5]. These new studies need a sensitive, accurate, and rapid method of analysis in human plasma so that the bioavailability and bioequivalent studies can easily be performed as per guidelines of FDA. The technique used in this study was spiking of plasma *in-vitro* thus omit the need of volunteers for the sampling. This step helps in making the study rapid. Various studies have been performed on the SDPAA using HPLC and spectrophotometric method [2,6-12].

SDPAA has been found to be effective against all strains of multidrug-resistant *E. coli*, with a MIC of 25 micrograms/ml [3,5]. Therefore, SDPAA may have the capacity to treat uncomplicated urinary tract infections caused by *E. coli*. It has also been shown to be effective in treating *Salmonella* infections in mice, and is under investigation for the treatment of tuberculosis [4].

SDPAA may also be a unique member of NSAID as it inhibits the lipoxygenase pathway and reduces formation of the leukotrienes (also pro-inflammatory autacoid). It is also involved in the inhibition of phospholipase A_2 [13]. It is well absorbed in GIT when given orally in form of tablets. It shows extensive hepatic metabolism. Metabolites form as glucronide and sulphate conjugates. Its peak appears in 30min and show half life of 1-2hr. The volume of distribution is 1.4 L/Kg and is about 99% protein bound. It also appears in synovial fluid after oral absorption. It is excreted in urine and from gut as glucronide and sulphate conjugates [8].

For detection of SDPAA using several HPLC methods in various body fluids SDPAA including blood, plasma, urine, synovial fluid SDPAA etc. have been carried out [6]. Each procedure has some advantages and some disadvantages. Extensive work has been performed on SDPAA in various biological solutions / fluids SDPAA including urine, plasma, bile, synovial and cranial fluids. Both HPLC and Spectrophotometric analysis have been made on the individual drug and as a comparison with other drugs [10,12,14-16].

A simple, rapid, and sensitive HPLC method for determination of SDPAA in human plasma was developed and one-step extraction of SDPAA was carried out by protein precipitation using ACN. This method can be used for determination of SDPAA concentration and pharmacokinetic and bioequivalence studies on both animals and human volunteers. UV-spectrophotometer method was also adopted to compare the results of HPLC method. Advantage of present study is to evaluate and compare the both HPLC and U-Spectrophotometric methods. In this work we have tried to develop low cost method by

minimum utilization of organic solvent in HPLC method. Moreover, we have also compared our HPLC results with UV-Spectrophotometric results to find out which method is more reliable, simple, precise, rapid, robust and accurate.

2. MATERIALS AND METHODS

SDPAA was obtain from Novartis Pharmaceuticals (Karachi, Pakistan). ACN and Methanol of HPLC grade was purchased by Merck (Germany). Sodium dihydrogen phosphate and sodium hydroxide of laboratory grade were purchased by Merck (Germany). Human plasma was obtained from blood bag Code No. CPDA-1 containing A+. Each 100 ml carry citric acid, Na-citrate, monobasic $NaPO_4$, dextrose, and adenine. The chemicals were obtained from the college laboratory as well as from the serveier pharmaceuticals.

2.1 Preparation of Phosphate Buffer (pH 6.8)

Add 0.60 ml of 1 M disodium hydrogen phosphate into the 4.4 ml of 1 M sodium dihydrogen phosphate to make the pH 6.8 and make the volume to 45 ml with distilled water. Adjust the pH to 6.8. For a higher pH add NaOH and for a lower pH add Ortho-phosphoric acid (H_3PO_4). Make the final volume upto 50 ml.

2.2 Preparation of Mobile Phase

Take 400 ml of ACN in 1 L volumetric flask. Add 300 mL of ACN in phosphate buffer of pH 6.8. Shake it gently and then the phosphate buffer is added up to the mark. Filter the solutions after the mixing by using micro-size membrane filter paper.

2.3 Preparation of Standard Stock Solution

Weigh exactly 10 mg of SDPAA and put it in a pre-washed 100 ml volumetric flask. Add 30 ml of previously filtered Methanol in volumetric flask and sonicate it for 2 min so that the drug get dissolve. Now Methanol was added up to the mark. This will give the strength of 0.1 mg/mL or 100 µg/ml.

2.4 Preparation of Standard Solution (without plasma)

Take the standard stock solution and add in it the mobile phase in such a way that a dilution of 0.05, 0.1, 0.5,1,2,5,10,15 and 20 µg/ml were achieved. Dilutions were prepared as follow:

Take 1 ml of stock solution in a test tube and make the volume up to 10ml by adding the mobile phase. It will give a dilution of 10 µg/ml. The rest of dilutions were prepared the as given in Table 1.

2.5 Experimental Procedure

2.5.1 Extraction of plasma from blood

Human plasma was obtained from blood bag Code No. CPDA-1 containing A+. 10 ml blood having EDTA was drawn from the bag and plasma was separated from it by centrifugation of the blood at 4500 rpm for 10min. The blood cells settle down and the supernatant plasma separated was subjected to the following procedure.

2.5.2 Spiking of drug in plasma and extraction of drug from plasma

1ml of plasma was added to 1ml of each standard solution separately and 0.5ml of buffer (pH 6.8) was added and vortex it for 30 seconds, and then added 0.5 ml of ACN. This will help in precipitation of plasma protein. Now vortex it for 2 min. Then centrifuge the mixture for 9 min at 4500 rpm. Separate the supernatant, filter it and finally analyze it by using following instruments.

1. UV-spectrophotometer.
2. Inject 1.5 ml of this supernatant to the HPLC.

Take the absorption spectra and relevant peaks and then interpret the results.

2.5.3 General method validation and its parameters by hplc

Once the method is developed it has to be validated. Method validation and development are part of each other. Without validating the procedure a new method cannot be consider to be a reliable one and need some changes or not. If a step is supposed to be changed it has to be revalidated. To avoid repeated validation studies a sequence of steps have been established.

2.5.4 Specificity/selectivity by HPLC

The terms are used interchangeably but specific term is used for a particular analyte while selectivity is used for the method in which closely

Table 1. Dilution pattern of standard stock solution for preparation of calibration solutions

Sr. No.	Amount of SDPAA stock solution (ml)	Amount of mobile phase added (ml)	Final concentration/ dilution(µg/ml)
1.	2	8	**20**
2.	1.5	8.5	**15**
3.	1	9	**10**
4.	0.5	9.5	**5**
5.	0.2	9.8	**2**
6.	0.1	9.9	**1**
7.	0.05	9.95	**0.5**

related compounds show responses. The term specificity describes the degree to which an analyte of interest is measured in the presence of other analyte/compound [17].

2.5.5 Precision by HPLC

It is the extent to which the scattered data agree to the measured standard deviation and is measured as RSD. It is further divided into the repeatability, reproducibility and intermediate precision. Repeatability means when the test is performed by same person on same instrument on same day but for 6 times and gives same results. Reproducibility means when the 6 samples are analyzed on different days or in different labs or by different instruments and give similar results. Intermediate precision is the analyses performed by different analyst, on different instruments, on different days in multiple labs [18].

2.5.6. Accuracy by HPLC

It is the measure of the errors both systemic and random from the data. Accuracy gives the idea that how closely the method can give assay value to the test value. It is usually determined by recovery studies.

2.5.7 Linearity by HPLC

It is the ability to obtain results that are directly proportional to the analyte concentration. in sample. Normally 5 values are enough to detect the curve in data; however, a long range of data gives more confidence on linearity studies. It is measured as a regression co-efficient of the data [19].

2.5.8 Robustness by HPLC

It is the effect of changes in the physical parameter of the methods on the result. The physical parameter includes changes in pH, buffer, mobile phase, temperature and solvent [20].

2.5.9 Administration of commercial brand to human volunteer

A commercial brand of SDPAA was given to a healthy volunteer who has not taken any analgesic since past two weeks. The blood samples was drawn after 2 hour of ingestion. The drug was extracted by the proposed method and the chromatogram was analyzed.

3. RESULTS AND DISCUSSION

3.1 Concentration of SDPAA with Plasma

The concentration of SDPAA in standard solution (with plasma), peak area and retention time (min) are shown in Table 2. The calibration curve between concentrations against peak area of SDPAA with plasma is shown in Fig. 1. The chromatograph of standard containing 20 µg/ml of SDPAA extracted with plasma is shown in Fig. 2.

3.2 Concentration of SDPAA without Plasma

The concentration of SDPAA in standard solution (without plasma), peak area and retention time (min) are shown in Table 3. The calibration curve between concentrations against peak area of SDPAA without plasma is shown in Fig. 3. The chromatograph of standard containing 50 µg/ml of SDPAA without plasma is shown in Fig. 4.

3.3 Percentage Recovery of SDPAA from Plasma

The percentage of SDPAA extracted from the plasma is calculated by comparing the peaks of the extracted SDPAA and standard solution of same conc. The %age recovery is calculated as:

% age purity =

$$\frac{\text{Peak area of sample x Conc. of standard}}{\text{Peak area of standard conc. of sample}} \times 100$$

3.4 Validation of Chromatographic Analysis

3.4.1 Linearity by HPLC

The linearity curve was drawn between various concentrations of standard solution extracted from plasma and their peak areas as shown in Fig. 5. The sample solutions, extracted from plasma were subjected to the chromatographic column and a peak for every concentration was taken and graph is plotted between the concentration and peak area. The results were linear between a range of 0.5–20 µg/ml with a regression equation of Y= 0.0477X + 0.0403 and R^2 =0.9991.

3.4.2 Limit of detection by HPLC

The limit of detection was calculated visually from the chromatograph. A series of dilution were prepared and extracted from plasma ranging from 0.02–0.5 µg/ml. The LOD observed was at concentration of 0.02 µg/ml (shown in Fig. 6).

3.4.3 Limit of quantitation by HPLC

The limit of quantitation was calculated visually from the chromatogram. A series of dilution were prepared and extracted from plasma ranging from 0.02–0.5 µg/ml. The LOQ observed was at concentration of 0.5 µg/ml (shown in Fig. 7).

3.4.4 Precision by HPLC method

The samples were analyzed as triplicates on intraday at different time intervals to check the precision of the method. The results showed the average % RSD as 1.34% as shown in Table 5. The results of inter-day precision performance shown in Table 5.

3.4.5 Accuracy by HPLC

The accuracy test was performed by making a solution of 10ug/ml of SDPAA (extracted from

Table 2. Concentrations of SDPAA with plasma, their respective peaks and retention time

Sr. no.	Concentration of SDPAA in standard solution (with plasma) (µg/ml)	Peak area (mAU)	Mean retention time (min)
1.	0.5	5010	5.669
2.	1	11188	
3.	2	22965	
4.	5	60780	
5.	10	123318	
6.	15	189719	
7.	20	252700	

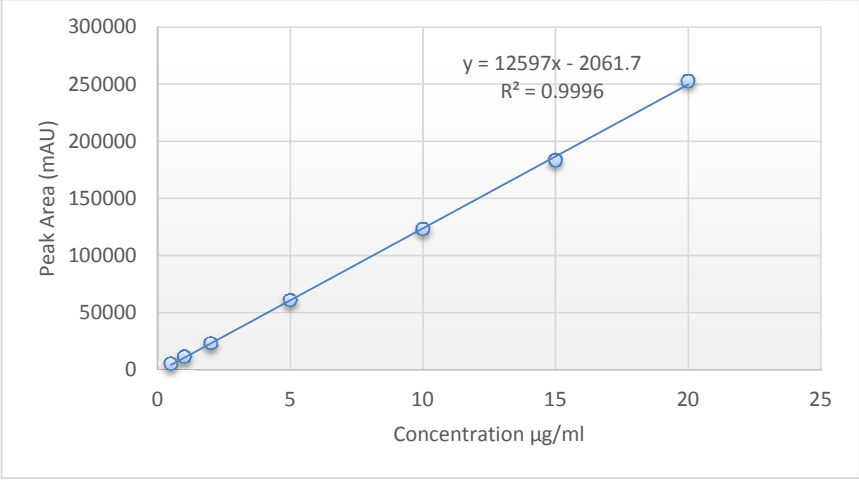

Fig. 1. Calibration curve between Concentration VS Peak Area of SDPAA in plasma

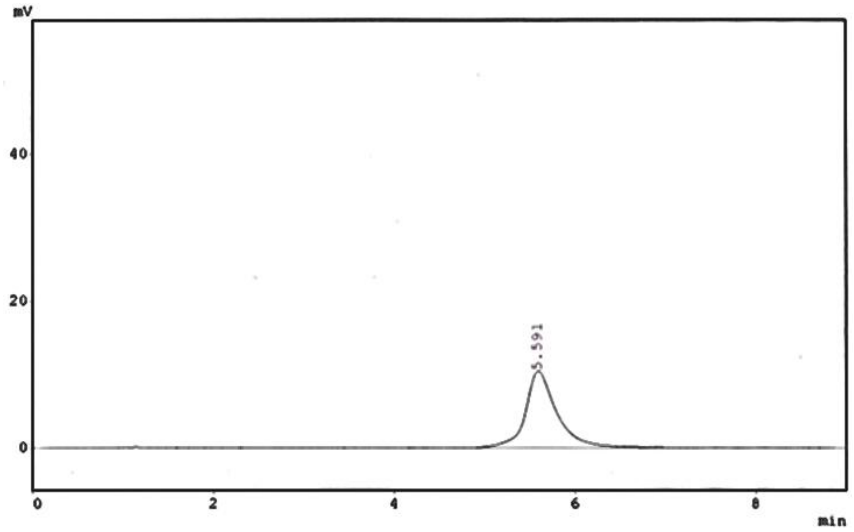

Fig. 2. Chromatograph of sample containing 20 µg/ml of SDPAA extracted from plasma

Table 3. Concentrations of SDPAA extracted without plasma, their respective peaks and retention time

Sr. No.	Concentration of SDPAA in standard solutions (extracted without plasma) (µg/ml)	Peak area (mAU)	Mean retention time (min)
1.	0.5	6006	5.667
2.	1	11146	
3.	2	22897	
4.	5	61194	
5.	10	119317	
6.	15	184631	
7.	20	262711	
8.	50	322,902	

plasma) at three %age levels 96.82%, 95.29% and 99.63% to check the efficiency and accuracy of method. A recovery range of 95.29% to 99.6% was observed. Results are shown in Table 6.

3.4.6 Recovery test by HPLC

To check the recovery test of proposed method a commercial brand of SDPAA mainly Voltral 50mg was given to healthy individual who has not taken any analgesic for past two weeks. The blood sample was taken after two hours of ingestion. The drug was extracted as per proposed method and was subjected to HPLC column for observing the peak. The resulting peak was super imposable to the peak obtained with standard conc. of 20 µg/ml. Chromatograph of sample obtained after 2 hr of ingestion of drug by a healthy volunteer as shown in Fig. 8. The recovered %age of injected sample of SDPAA is shown in Table 6a. Hence, this proves that our proposed method is accurate, precise and more efficient than previously reported works.

3.4.7 Retention time in HPLC

The average retention time for the SDPAA without plasma was 5.669 min while the samples extracted from the plasma showed an average retention time of 5.667 min.

3.4.8 Robustness by HPLC

The robustness validation has been performed by changing different conditions to evaluate occurrence of any changes in developed method. The results were under limits as shown in Table 7.

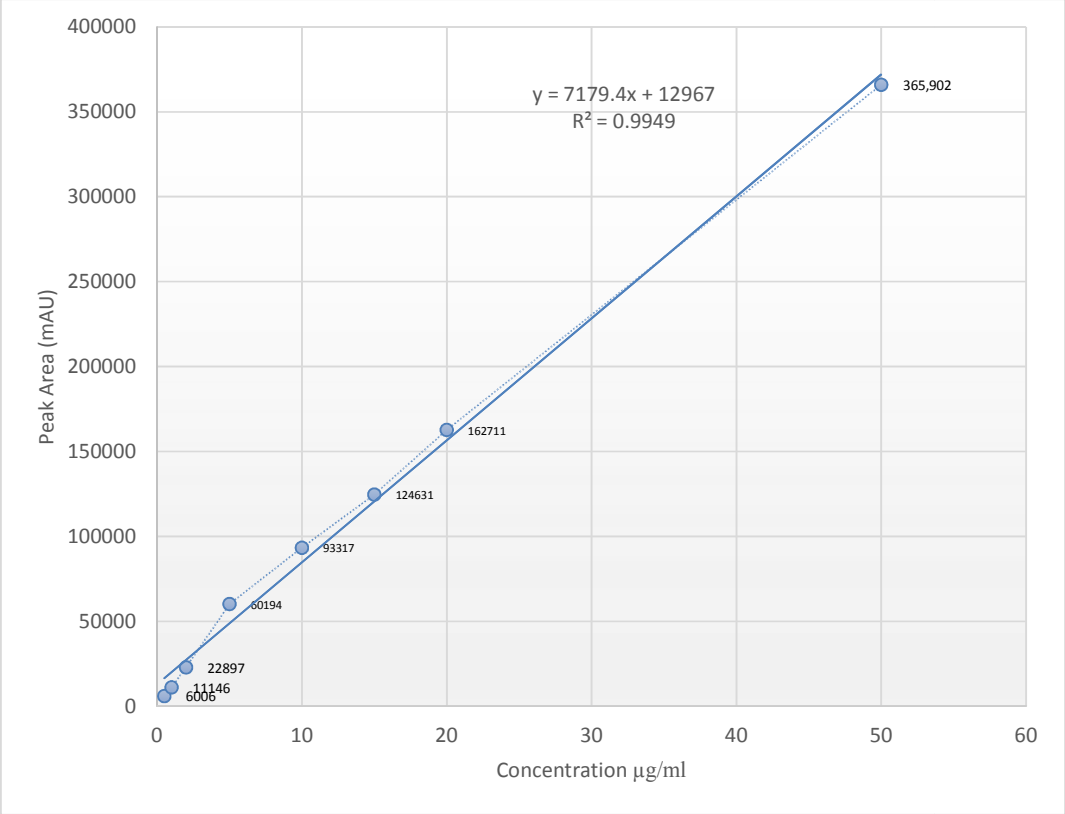

Fig. 3. Calibration curve between concentrations versus peak area of SDPAA without plasma

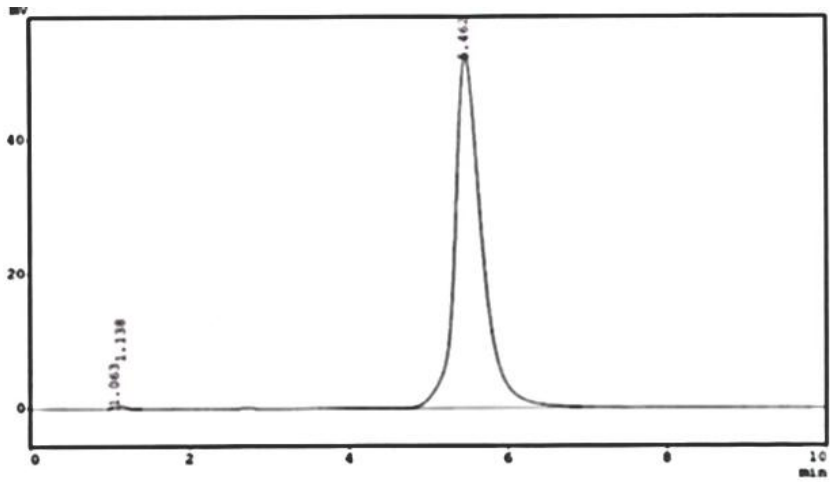

Fig. 4. Chromatograph of sample containing 50 µg/ml of SDPAA without plasma

Table 4. Percentage recovery of SDPAA from plasma

Conc. of std. soln (without plasma) (µg)	Peak area of std. soln (without plasma)	Conc. of sample (extracted from plasma) (µg/ml)	Peak area of sample (extracted from plasma)	%age purity
2	22965	2	22978	102%
5	60800	5	60796	98.9%
10	123318	10	123318	100%

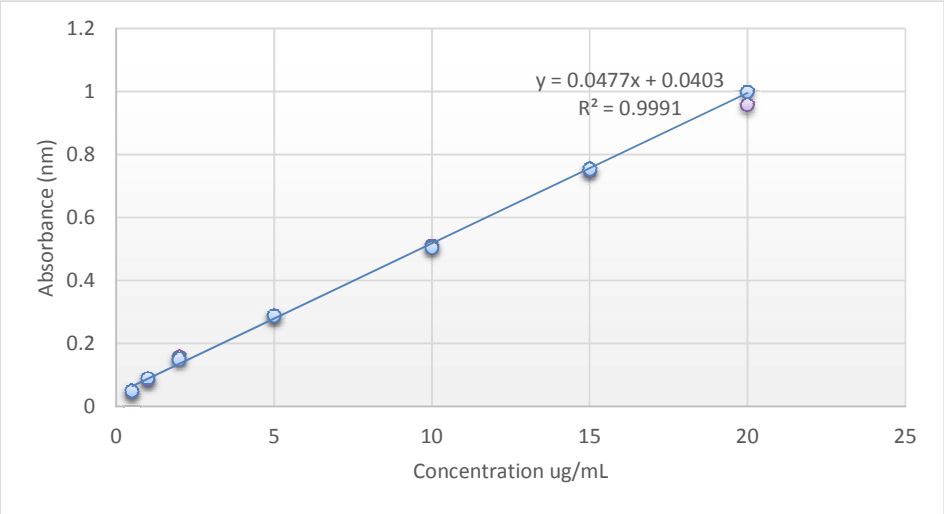

Fig. 5. Linearity curve was drawn between various concentrations of standard solution extracted from plasma and their peak areas

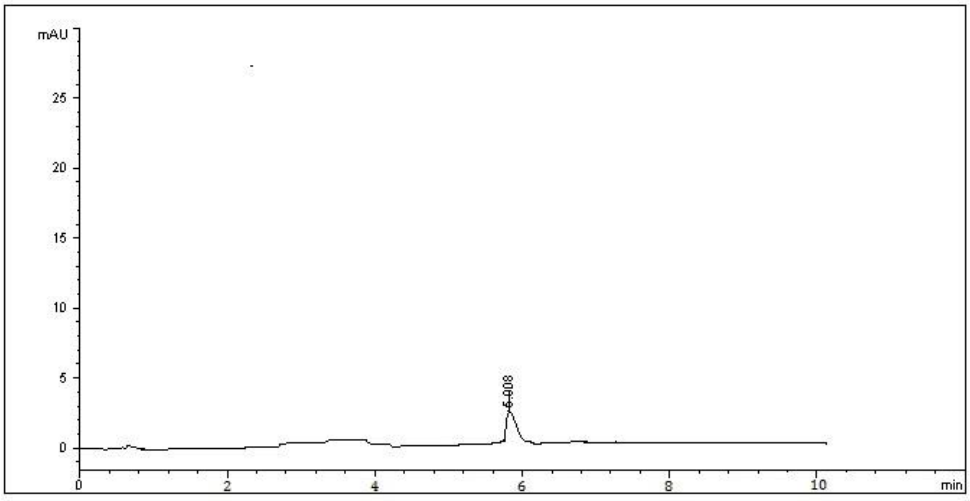

Fig. 6. Chromatograph of LOD observed at concentration of 0.02 µg/ml at retention time 5.908 min

3.5 Analysis of SDPAA by Spectro-photometric Method and Its Validation

3.5.1 Specificity/Selectivity

For spectrophotometric analysis the lambda max is observed using blank as reference. A solution mixture of ACN: buffer (pH = 6.8) 40:60 was used as a blank to set the base line. Now a standard solution of 10ug/ml without plasma was taken in the quartz cell to observe the absorption spectra. A peak was observed at a wave length of 281nm (results shown in Fig. 9).

3.5.2 Linearity by UV-Spectrophotometer

Each standard solution was taken one by one and its corresponding absorbance was checked at a wavelength of 281nm. The baseline was made using solvent mixture as blank. A curve was drawn between the concentration and its respective absorbance where the concentration was taken at X-axis and

absorbance at Y-axis. Results are shown in Table 8 and Fig. 10.

3.5.3 Limit of detection and limit of quantitation by uv-spectrophotometer

The samples were prepared and extracted from plasma in six replicates. Each sample was subjected to the spectrophotometer and linearity equations were obtained. A mean of slopes and standard deviation of Y-intercept was calculated and L.O.D was calculated which was 0.03 µg/ml. Similarly, a mean of slopes and standard deviation of Y-intercept was calculated and L.O.Q was calculated which was 0.109 µg/ml.

3.5.4 Accuracy by UV-Spectrophotometry

The samples obtained after the extraction from plasma were compared with the standard solutions of same strength without plasma to check the recovery and efficiency of the method. The results are shown in Table 9.

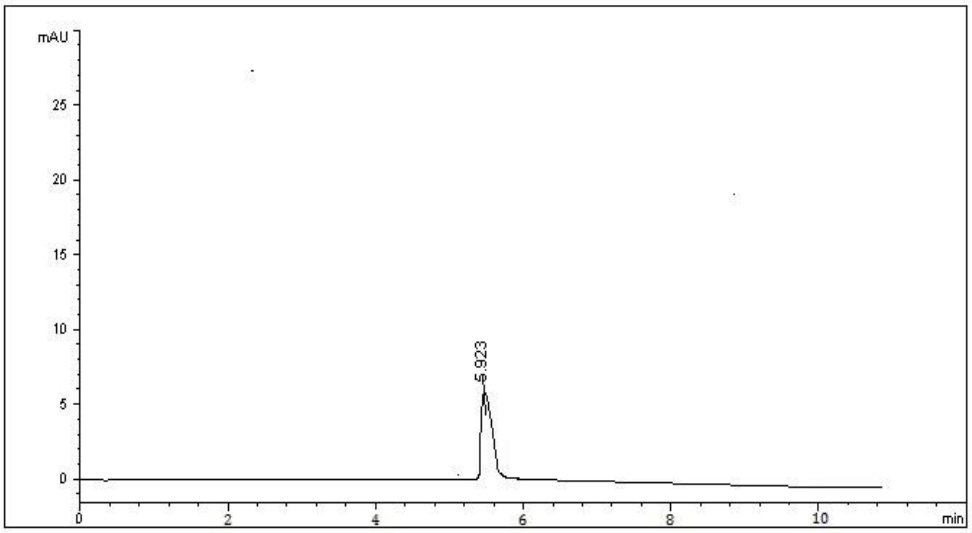

Fig. 7. Chromatograph of LOD observed at concentration of 0.5 µg/ml **at retention time 5.902 min**

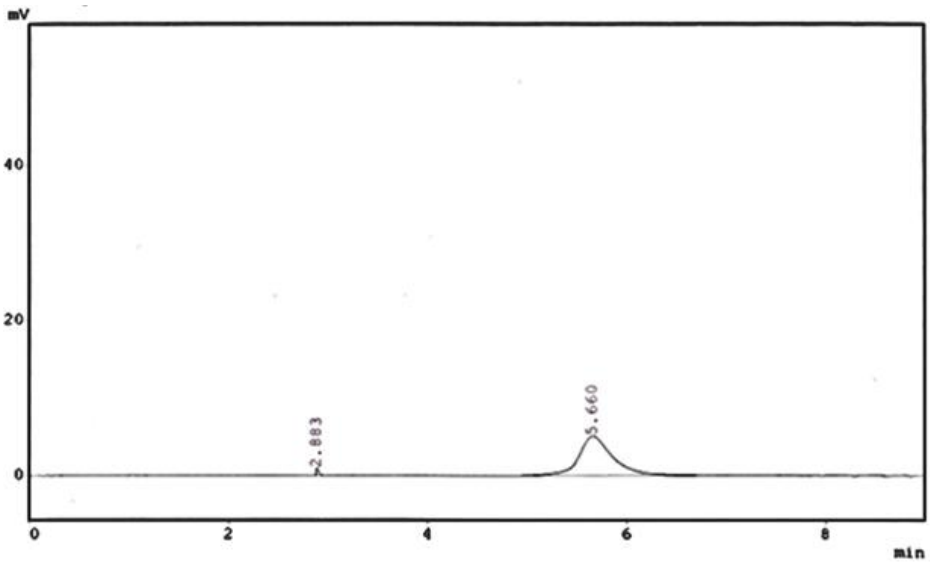

Fig. 8. Chromatograph of sample obtained after 2hr of ingestion of drug by a healthy volunteer

Table 5. Intraday and Inter-Day analysis of 10 µg/ml of extracted SDPAA by HPLC method

Intra-day analysis					
Sr. No.	**Time (hrs)**	**Wavelength (nm)**	**Concentration (µg/ml)**	**Retention time (min)**	**Peak Area (mAU)**
1.	0	281	10	5.472	1254847
2.	1	281	10	5.447	1235282
3.	2	281	10	5.470	1207844
4.	4	281	10	5.467	1247423
5.	6	281	10	5.462	1245756
6.	8	281	10	5.461	1243186
	Mean			5.46	1239056
	Standard Deviation				16551
	% RSD				1.34
Inter-day analysis					
Sr. No.	**Days**	**Wavelength (nm)**	**Concentration (µg/ml) (n=3)**	**Retention time (min) Mean (n=3)**	**Peak Area (mAU) Mean (n=3)**
1.	Day 1	281	10	5.434	1207844
2.	Day 2	281	10	5.412	1247423
3.	Day 3	281	10	5.443	1245756
	Mean			5.429	1233674
	Standard Deviation				16923
	% RSD				1.39

Table 6. Accuracy test at three %age levels of SDPAA

Assay no.	Conc. of sample (µg/ml)	Peak area of sample	%age of sample at levels	Peak Area (mAU)
1	10	123318	99.63	1251624
2	10	123318	96.82	1216315
3	10	123318	95.29	1197185
Mean			97.25	1241415
Standard deviation				21862
%RSD				1.76

Table 6a. Recovered %age of injected sample of SDPAA

Peak area of standard solution (extracted from plasma)	Conc. of standard solution (extracted from plasma)	Peak area of volunteer sample (extracted from plasma)	%age recovered
252700	20ug/ml	251873	99.6

$$\text{Conc. Recovered} = \frac{\text{Peak area of sample x Conc. of standard}}{\text{Peak area of standard x \% age recovered}} \text{ X } 100$$

$$= \frac{251873 \text{ x } 20}{252700 \text{ x } 99.6} \text{ X } 100$$

$$= 19.99 \text{ µg/ml}$$

3.5.5 Precision by UV-Spectrophotometry

The SDPAA samples were analyzed as on intraday and inter-day to check the precision of the method. The results showed the average %age RSD was less than 2% which is acceptable. Average %RSD is about 0.41. Results are shown in Table 10 to 11.

Table 7. Evaluation of robustness with deliberate changing in different variables

No. of injection	Peak area of drug		STDEV		%RSD	
	(Without plasma)	With plasma	Without plasma STDEV	With plasma STDEV	Without plasma % RSD	With plasma % RSD
			Flow Rate : 1.25			
1	5501796	554425	3168.152	2544.440	0.541	0.310
2	541481	541806				
3	543166	547212				
			Flow Rate : 1.75			
1	336653	327065	2438.016	4566.680	0.507	1.109
2	331400	343481				
3	324526	348256				
			Change of Mobile Phase pH : 3.2			
1	472068	475397	3343.314	5015.943	0.509	1.029
2	437071	483919				
			Change of Mobile Phase pH : 3.7			
1	402449	424315	385.172	3445.077	0.078	0.797
2	401890	403972				
			Change of Column (18-25 ℃)			
1	421930	442005	3454.035	1316.026	0.359	0.402
2	446464	459861				

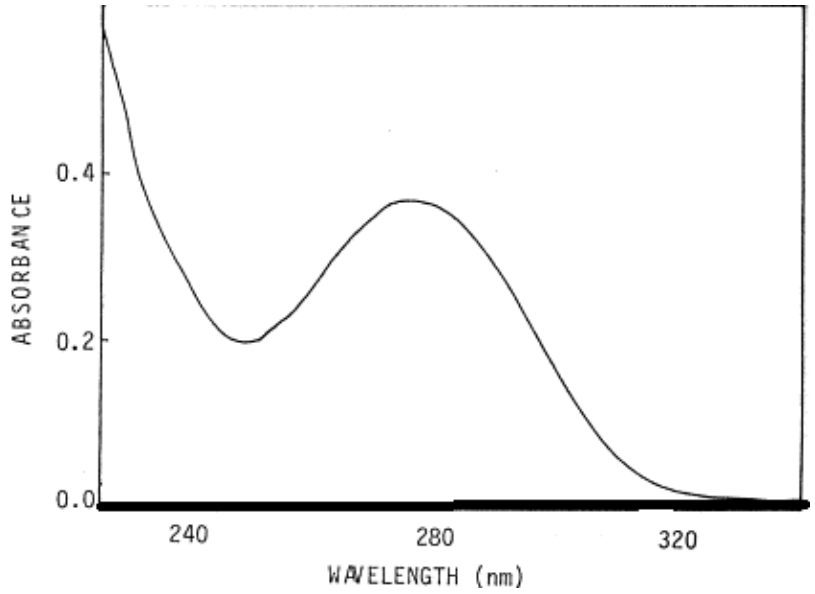

Fig. 9. Spectrum of SDPAA (10 µg/mL) in ACN:Buffer Solvent

Table 8. Concentrations of SDPAA without plasma and the relative absorbance observed

Sr. no	Concentration of SDPAA in standard solutions (without plasma) (µg/ml)	Absorbance at 281 nm (nm)
1.	0.5	0.046
2.	1	0.085
3.	2	0.158
4.	5	0.289
5.	10	0.510
6.	15	0.750
7.	20	0.957

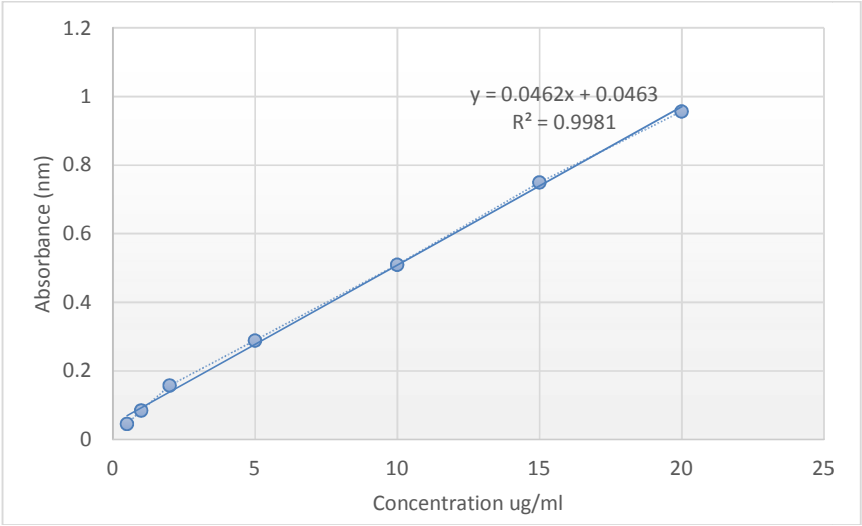

Fig. 10. Linearity curve of different concentrations of SDPAA and the relative absorbance by UV-Method

Table 9. % age recovery of SDPAA from the spiked plasma

Sr. No.	Concentration of solution (µg/ml)	Absorbance in standard	Absorbance in plasma	%age purity
1	2	0.158	0.151	95.569
2	5	0.289	0.288	99.653
3	10	0.510	0.505	99.01
4	15	0.750	0.754	100.53
5	20	0.957	0.999	104.30

Table 10. Intra-Day analyses of 1 µg/ml /ml, 2 µg/ml and 10 µg/ml of extracted SDPAA by spectrophotometric method

Sr. No.	Wavelength (nm)	Time (Hr)	1µg/ml Absorbance (nm)	2µg/ml Absorbance (nm)	10µg/ml Absorbance (nm)
1.	281	0	0.089	0.151	0.505
2.	281	2	0.089	0.151	0.505
3.	281	4	0.089	0.151	0.505
4.	281	6	0.089	0.151	0.505
5.	281	8	0.088	0.153	0.505
6.	281	10	0.088	0.153	0.508
Mean			0.088833	0.15166	0.5055
Standard Deviation			0.000408	0.001033	0.001225
% RSD			0.4597	0.6809	0.242284

Table 11. Inter-day analyses of 15 µg/ml extracted SDPAA by spectrophotometric method

Sr. No.	Wavelength (nm)	Mean absorbance (nm)	Standard deviation	% RSD
Day 1	281	0.754	0.002041	0.2704
Day 2	281	0.754	0.002041	0.2704
Day 3	281	0.754	0.002041	0.2704
Week 1	281	0.754	0.002041	0.2704
Week 2	281	0.7576	0.0010	0.136
Week 3	281	0.758	0.001835	0.242

4. DISCUSSION

The validation by HPLC has becoming much important in these days as it is considered to be more sensitive and accurate method. The objective of this study was to develop and validate a rapid, precise, accurate and sensitive method for determination of SDPAA from human plasma. For this reason an HPLC method was developed and findings were compared with the results of spectrophotometric method. No significant differences were observed between findings of both methods. The non-significant differences were observed between recovery concentration of SDPAA from plasma and without plasma samples which proves authentication of reproducible HPLC method. Calibration curves in both situations (with and without plasma) showed acceptable findings.

Validation of method was confirmed by different processes. The precision test were performed on inter-day and on intra-day the results were reproducible and the average RSD was less than 2% which is within acceptable range. The %age recovery of SDPAA from plasma was within 96-101.5% which is also acceptable. Accuracy was confirmed with the recovery studies and by three test assays. Accuracy was checked at three %age levels and was within 95–99%. The LOD was 0.02 µg/ml and LOQ was 0.5 µg/ml. The LOD and LOQ were observed visually using signal to noise ratio.

Table 12. Difference between results of HPLC and UV-Spectrophotometry

Parameters	HPLC	UV
Linear range (µg/ml)	0.5-20	2-22
Coefficient Regression (R^2)	0.999	0.998
Precision (RSD %)	0.24	1.3
LOD (µg/ml)	0.02	0.03
LOQ (µg/ml)	0.5	0.109

After oral administration of SDPAA to human volunteer, result showed 19.9 µg/ml recoveries as shown in Table 1. Thus, this proves that the proposed method is accurate and efficient. The linearity curve using spectrophotometric method was drawn using a six dilutions i.e., 0.5, 1, 2, 5, 10, 15 and 20 µg/ml of SDPAA which was extracted from the plasma. In spectrophotometry, a linear range of 0.5-20 µg/ml was observed with the regression equation Y=0.0462X+0.0463 and the R^2 was 0.998 which was in acceptable range. The sensitivity, precision, accuracy, limit of detection and limit of quantitation analysis were also performed by UV-spectrophotmeter and findings were within limit. After comparison of both results of both methods, results were found with less differences between them. The comparisons of few parameters regarding HPLC and UV-spectrophotometer are shown in Table 12.

5. CONCLUSION

The proposed UV-spectrophotometric and HPLC method were found as very simple, rapid and economical techniques for the determination of SDPAA from plasma and without plasma and comparative evaluation of results achieved by both techniques. The methods are validated in compliance with US-FDA guidelines is suitable for estimation of SDPAA with excellent accuracy, repeatability, recovery, precision and linearity.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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