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DERIVATIVE ULTRA-VIOLET SPECTROSCOPIC METHOD FOR THE ESTIMATION OF CERBERIN IN RAT PLASMA

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ABSTRACT

Cerberin (2-o-Acetyl neriifolin) is the principal cardiac glycoside present in seeds of *Cerbera odollam*.The seeds of *Cerbera odollam* are used as a poison for suicidal as well as homicidal purpose by people around the world. Its detection in body fluids is somewhat difficult .The present study aims to develop a simple Ultra Violet derivative spectroscopic method for the estimation of cerberin from rat plasma without previous chemical separation using fourth derivative of the zero-order absorption spectra and the integrated area of the peak (valley) ranging from wavelength 305.2 to 307.2 nm.

KEYWORDS: Cerberin, derivative, rat blood plasma, UltraViolet spectroscopy

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INTRODUCTION

Cerbera odollam is a tree belonging to the poisonous Apocynaceae family[1,2], where the seeds of the species are found to contain toxic principle Cerberin as the main active cardenolide[3,4] . *Cerbera venenifera*, a related species found in Madagascar, has a long history as an ordeal poison, and was responsible for the death of 3000 people per year in previous centuries[5] The *Cerbera odollam* tree is responsible for about 50% of the plant poisoning cases and 10% of the total poisoning cases in Kerala, India[6] It is used both for suicide and homicide. It is a powerful toxic plant that is currently completely ignored by western physicians, chemists, analysts and even coroners and forensic toxicologists[7].Even it can be taken to other countries where it is not grown for murdering. Cytotoxic activity of cardenolide principle from the seeds of *Cerbera odollam* (Image.1) has been studied.[8]The Burmese use it for lighting, as a cosmetic, or mixed with other oils as an insecticide or insect repellent[9].Cardiac properties in Cat shows a rise in blood pressure and decrease in heart rate[10] .Triticusterol,2,6-Dihydroxy-4-methoxy benzoic acid,2-Hydroxy-4-methoxy-6-methyl benzoic acid has been isolated from the stem bark of *Cerbera odollam.* Literature Review shows that only one method is reported so far for the determination of Cerberin by UPLC-MS method [11]. Therefore separation techniques generally have to be used to allow the robust detection of the analytes. The structure of Cerberin (Figure.1) makes difficult for their assay in mixtures and even more difficult in complex matrices such as biological fluids or tissues, mainly if low-cost methods are available. Derivative spectrophotometry (DS) is such a technique used to discriminate and allow the assay of certain analytes from complex mixtures or matrices *via* mathematical interpretation of the absorption signal. It is based on the so called *derivate spectra* which are generated from parent zero-order ones.[12] The derivatisation of zero-order spectrum can lead to separation of overlapped signals and/or the elimination of background caused by presence of other compounds in a sample[13,14] .Nowadays, this technique found application in many fields of analysis, mainly in the pharmaceutical, clinical and biochemical one as well as in inorganic or organic analysis[15] The study aims to develop a derivative UltraViolet spectroscopic method for the assay of Cerberin at low concentrations from Rat plasma without chemical separation. The present study can be further used in analytical toxicology to find Cerberin concentration in human plasma in cases of accidental intoxications or suicidal purpose.

Figure 1
Structure of Cerberin

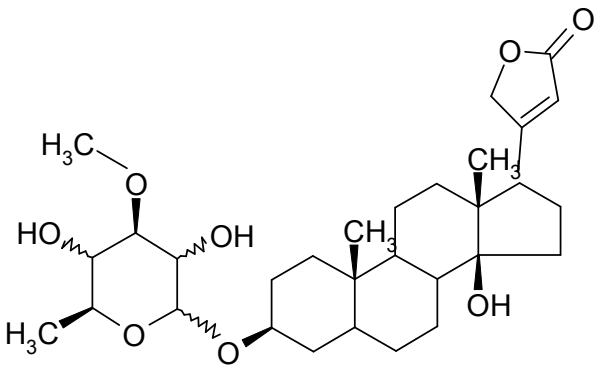


Image 1
Fruit exposed to show seed

MATERIALS AND METHODS

Reagents
- Cerberin-Pure standard from Sigma Aldrich –
Stock solution of 100 µg/mL in methanol
- Methanol, HPLC grade, from Merck, was used as solvent in all experiments.
- Rat plasma was obtained from KMCH College of Pharmacy, Coimbatore.

Instruments
Shimadzu UV-VIS spectrophotometer, UV Probe 2.1 Version software. Shimadzu Electronic balance AW 220,Centrifuge Kemi 5600.

Method
Zero order spectrum of different concentrations of Cerberin in rat plasma was subjected to derivatization up to fourth order by the UV Probe software.2.1 version.

Procedure
The Rat plasma was diluted 1:10 with double distilled water. Appropriate aliquots from the stock solution and of the diluted Rat plasma to get the desired concentration of Cerberin were pipetted in testing tubes and gently vortex-mixed for 7 minutes. A blank plasma sample was also prepared, containing the amount of methanol used for the samples. The UltraViolet absorption spectra of the above solutions were there after plotted against double distilled water in the range 200-400 nm, with a slit width of 2 nm and a scanning speed of 200 nm/min. Previously, a background correction of the device was performed with water.The zero-order spectra obtained were further derivatised (1-st to fourth derivatives) using the Facilities of the software (Mathematics mode) and used in the analysis[15,16].

RESULTS AND DISCUSSION

The zero-order spectra of the plasma containing 50-200µg/mL of Cerberin are presented in Figure.2. Beer's law plot for standard drug solution in methanol also showed in Table .1.

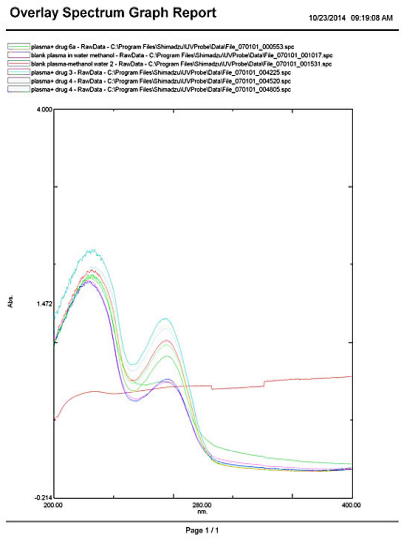
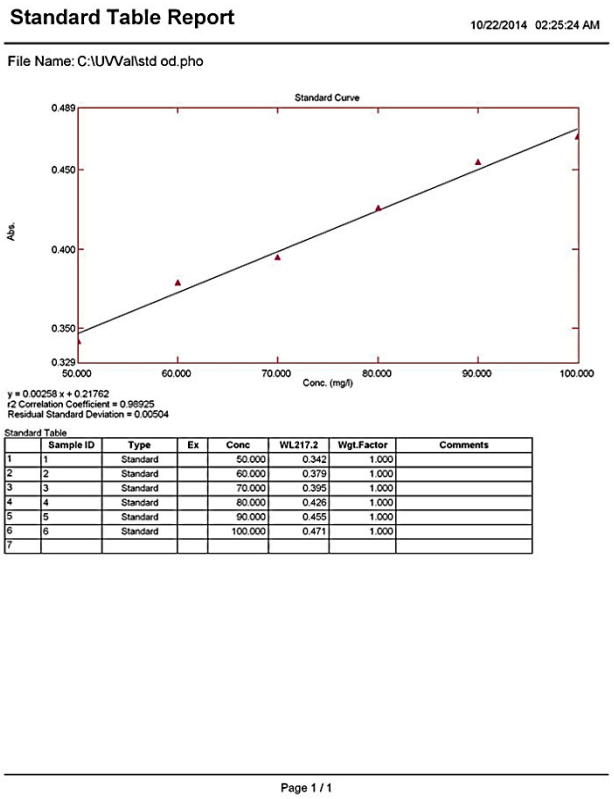


Figure 2
Zero order spectra of Cerberin in rat plasma



	Sample ID	Type	Ex	Conc	WL217.2	Wgt.Factor	Comments
1	1	Standard		50.000	0.342	1.000	
2	2	Standard		60.000	0.379	1.000	
3	3	Standard		70.000	0.395	1.000	
4	4	Standard		80.000	0.426	1.000	
5	5	Standard		90.000	0.455	1.000	
6	6	Standard		100.000	0.471	1.000	
7							

Table 1
Beer's law plot of Cerberin in rat plasma

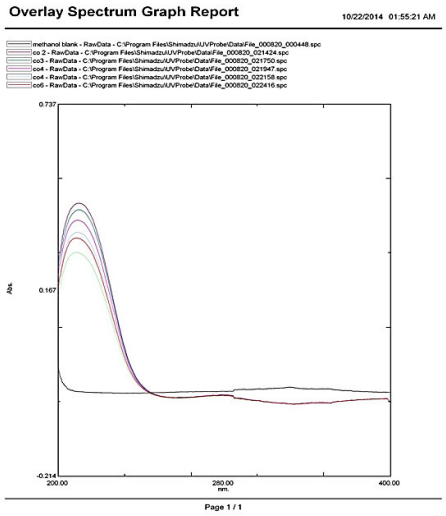
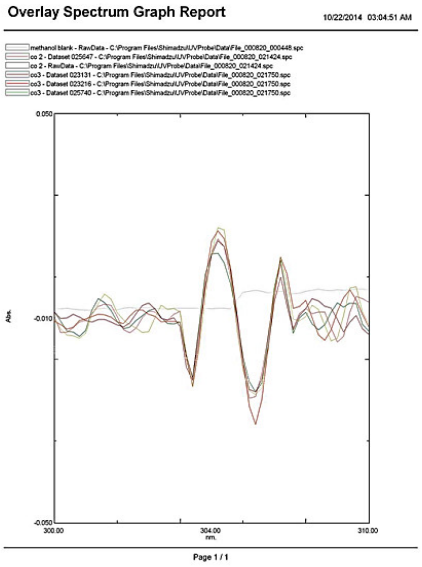


Figure 3
Overlay spectrum of Pure Cerberin

Certain differences between the samples can be observed in the range 250-350 nm(Figure.2), but the Pure drug in methanol solution showed Absorbance maxima at 217.2nm(Figure.3).Besides, the absorbance of the plasma spectrum from different animals can differ rather dramatically, and neither a standard addition method can be used due to the same small differences in absorbance at the limit of the instrumental error (0.001 absorbance units). The first to fourth derivatives of the spectra to look for zones in which the differences between the spectra are Interpretable

Figure 4
Fourth derivative spectrum of Cerberin



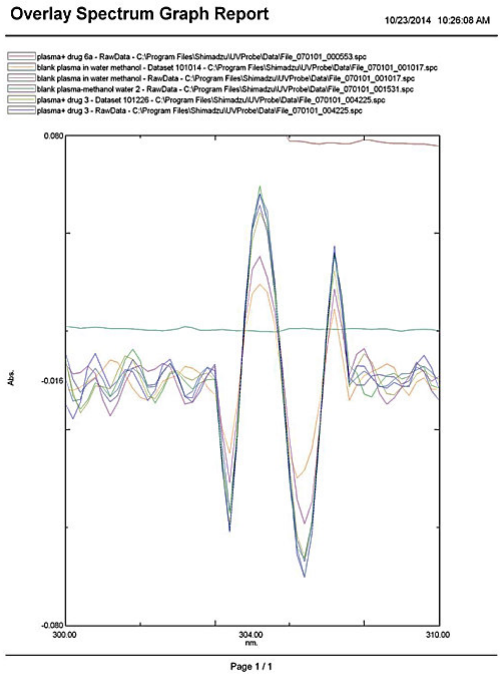


Figure 5
Fourth derivative spectrum of Cerberin in rat plasma

Figure.4 depicts the fourth derivative of standard drug solution and Figure. 5 depicts the fourth derivative of drug in rat plasma. As it can be seen, the derivatives are rather close to each other. However, in the region 300 – 310 nm of the fourth derivative a zone where the peaks (valleys) can be distinguished for each spectra and where the computational methods can be performed (i.e. a three point correction for the peak or an area of the peak can be computed, as all the spectra are intersecting at the same wavelength at one side and the other of the peak)

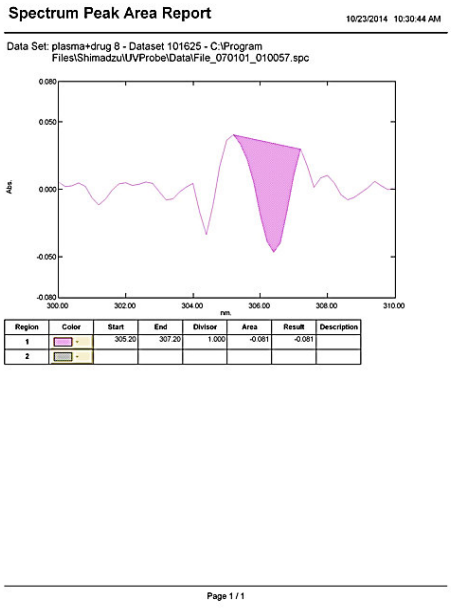


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Figure 6
Fourth derivative spectrum of Cerberin in Rat plasma

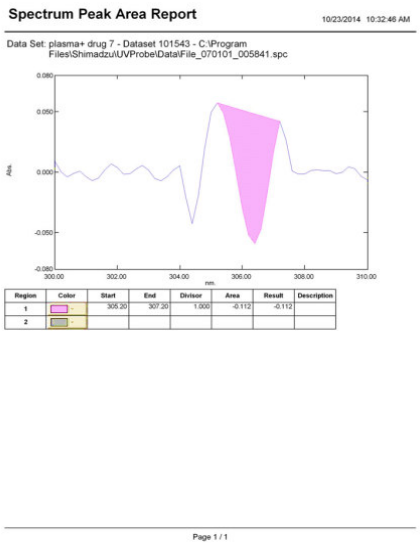

Figure 7
Fourth derivative spectrum of Cerberin in Rat plasma

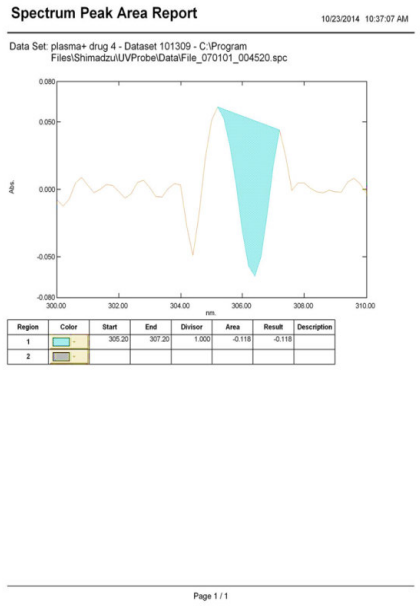

Figure 8
Fourth derivative spectrum of Cerberin in Rat plasma

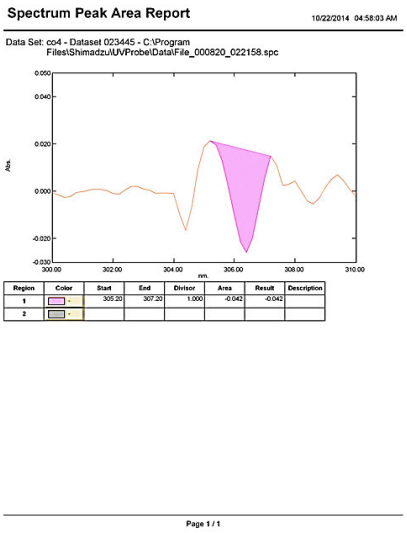

Figure 9
Fourth derivative spectrum of Cerberin in Rat plasma

As can be seen from Figure 6,7,8,9 the fourth derivatives of the spectra intersect at 305.2 and 307.2 nm, and have a peak (valley) at 306.2 nm. Regression analysis on the data coming from repeated measurements performed on 5 replications of each of the following Cerberin concentrations: 0 (plasma blank), 50, 60, 70, 80, 90 ,100 and 200µg/mL, using the peak value at 306.2 nm and the peak integrated value from 305.2 to 307.2 nm as a function of Cerberin concentration. The results are summarized in Table I.

Table 1
Assay of Cerberin in rat plasma using the 4thderivative of the UV spectrum

Parameter	Linear regression Equation	r^2	Standard error
Peak value	Y=-00014x-0.00771	0.9680	0.00052
Peak area	Y=-0.00044x-0.00490	0.9805	0.00341

Even if the correlation coefficients are quite close one to another (0.96807 for the peak value and 0.9805 for the peak area), the results are more reliable when using the area, as the influence of the background is cancelled by the fact that the blank sample and the Cerberin samples valleys are in the same range and the values on the ordinate scale are all of the same sign[17,18] (all negative). Therefore this method for the quantification of Cerberin in rat plasma without chemical extraction can be used for the assay of Cerberin in Analytical toxicology. The values obtained for testing the accuracy of the method resulted in a mean recovery coefficient of 99.7% for samples containing 50, 100 and 200µg/mL, (Table.2) which can be considered as acceptable for a simple and efficient method for toxicological monitoring of Cerberin in patients, taking into account that the toxic blood levels[19]for Cerberin range in the field 50µg/mL and lethal levels are above 300 µg/mL Of course, the method cannot replace methods as HPLC, GC, or LC-MS, but is far more simple, less time-consuming and less expensive than those.

Table 2
Recovery studies of Cerberin from rat plasma

Sample added in µg/mL	Sample recovered µg/mL	Percent recovered %	Mean Recovery
50	49.5	99	99.7%
100	101	101	
200	198.6	99.3	

CONCLUSION

The proposed method is a simple and versatile method for Cerberin quantitative assay in Rat blood plasma without separation of the drug from the biological matrix, using UltraViolet derivative absorption spectrophotometry. The method is based on the fourth derivative of the UVspectrum of blood plasma containing small amounts of Cerberin (ranging in the field 50-200 µg/mL), by integrating the area of the peak (valley) between 305.2 and 307.2 nm, where the derivatives are dependent of Cerberin concentration. The method can be useful to monitor the toxic blood level of Cerberin in order to avoid accidental or suicidal intoxication with Cerberin, mainly at the beginning of the treatment. The method cannot replace classical methods which are available such as HPLC or GC-MS , but is far easier to perform.

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