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Simultaneous Determination of Sulfacetamide and Prednisolone by H-Point Standard Addition Method with Simultaneous Addition of Both Analytes in Some Biological Fluids

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Simultaneous determination of sulfacetamide and prednisolone by UV-vis spectrophotometry and H-point standard addition method (HPSAM) with simultaneous addition of both analytes has described. The effect of various parameters including pH, wavelength selection and concentration ratio on the sensitivity and accuracy of method were investigated. The results of applying H-point standard addition method showed that sulfacetamide and prednisolone could be determined simultaneously with the concentration ratios of 1:6-10:1 in mixed samples, respectively. The method was applied to the simultaneous determination of sulfacetamide and prednisolone in some synthetic mixtures, plasma, whole blood and urine successfully.

Key Words: Sulfacetamide, Prednisolone, Spectrophotometry, H-Point standard addition method, Biological fluids.

INTRODUCTION

Sulfonamides are bacteriostatic agents and their spectrum of activity are similar for all[1]. Sulfonamides inhibit bacterial synthesis of dihydrofolic acid by preventing the condensation of the pteridine with aminobenzoic acid through competitive inhibition of the enzyme dihydropteroate synthetase. Topically applied sulfonamides are considered active against susceptible strains of bacterial eye pathogens. Prednisolone is in a class of drugs called steroids. Prednisolone prevents the release of substances in the body that cause inflammation. The combination of sulfacetamide and prednisolone is used to prevent or treat eye infections and treat swelling in the eye[2].

Corticosteroids suppress the inflammatory response to a variety of agents and they probably delay or slow healing. Since corticosteroids may inhibit the body's defense mechanism against infection, a concomitant antibacterial drug may be used when this inhibition is considered to be clinically significant in a particular case[3].

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When a decision to administer both a corticosteroid and an antibacterial is made, the administration of such drugs in combination has the advantage of greater patient compliance and convenience, with the added assurance that the appropriate dosage of both drugs is administered. When both types of drugs are in the same formulation, compatibility of ingredients is assured and the correct volume of drug is delivered and retained.

A sulfacetamide/prednisolone combination is indicated for steroid-responsive inflammatory ocular conditions for which a corticosteroid is indicated and where superficial bacterial ocular infection or a risk of bacterial ocular infection exists[3].

Sulfacetamide has been determined by liquid chromatography[4-8], solid phase micro extraction[9], spectrophotometry[10] and micellar electrokinetic chromatography[11].

In the case of prednisolone, several methods have proposed for determination of prednisolone base on liquid chromatography-tandem mass spectrometry[12-14], electrochemistry[15], gas chromatography-mass spectrometry[16] and liquid chromatography[17].

To our best of knowledge, sulfacetamide and prednisolone have been determined simultaneously in pharmaceutical dosage forms only by micellar electrokinetic chromatography[11]. Since UV-visible spectrophotometry is a rapid, sensitive and inexpensive analytical tool, it is appropriate for dosage control of pharmaceutical preparations. Despite the mentioned advantages, spectroscopy techniques (such as other analytical techniques) suffer from multiplicative (matrix effect) and additive (direct interference) errors. The problem of multiplicative errors can be simply solved by using standard addition method. The applicability of standard addition method is limited to the cases where no direct interference (additive error) is present. Additive errors are observed when two or more species in sample have spectral overlapping. In such cases determination of one analyte in the presence of interferent(s) by classical methods (*e.g.*, standard addition or external standard calibration) is not possible.

Various multivariate methods for handling nonselective signals in spectrophotometric analysis have been proposed. Principal component and partial least squares[18] solutions require no explicit data about the individual interferences to be known in order to model and to eliminate them. However, they require a set of calibration samples with known concentrations of the analyte and interferent in different samples to allow the model of algorithm calibration. In the most applications of multivariate chemometrics methods the matrix effects are not considered.

The generalized standard addition method[4] developed by Saxberg and Kowalski[19] is a multivariate extension of the conventional standard addition method for simultaneous multicomponent determinations. The GSAM looks promising as a method by which both interferences and the matrix effects can be overcome simultaneously. In this way, Falco *et al.*[20] proposed a modification of MOSA, H-point standard addition method, which makes it possible to determine the concentration of analyte

in the presence of a direct interferent and even the concentration of interferent can be determined. The basis of the method for spectrophotometric determination of binary mixtures with extensively overlapped spectra and in the presence of proportional errors was established[20,21]. This method has been frequently applied to improve results in different analytical techniques, including spectrophotometry[22-25], spectrofluorometry[26], kinetic specterophotometry[27-29]. chromatographic techniques[30] and stripping voltammetry[31]. Recently the method has used for spectral curve deconvolution in micellar system and spectrophotometric study of complexation equilibria[32,33].

In 1995 Falco *et al.*[34] proposed a modification of H-point standard addition method (HPSAM) with simultaneous addition of both analytes that permits the resolution of both species from a unique calibration set by making the simultaneous addition of both analytes. Two pairs of standard addition plots can be obtained by plotting the analytical signals in two pairs of selected independent wavelengths *versus* the added concentrations of analytes. Each pair of plots has an intersection point that its coordinate on the concentration axis is $-C_A$. The requirement of selecting only two pairs of wavelengths makes this method simpler to use in respect to GSAM.

In this work, the applicability of H-point standard addition method (HPSAM) with simultaneous additions of both analytes is verified to the resolving of overlapping spectra of sulfacetamide and prednisolone. The results show that HPSAM is suitable for the simultaneous determination of two species in aqueous media. The proposed method has successfully applied to the simultaneous determination of sulfacetamide and prednisolone in some synthetic samples, plasma, urine and whole blood with satisfactory results.

EXPERIMENTAL

All the chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany) unless stated otherwise. Doubly distilled water have used throughout. Sulfacetamide and prednisolone purchased from Behdashtkar and Sina Darou Medical companies (Tehran, Iran), respectively.

Solution of a 1×10^{-3} mol L^{-1} sulfacetamide was prepared daily by dissolving 0.0214 g sulfacetamide (99 %) in propanol-water (50:50, v/v) in a 100 mL volumetric flask. The solution was kept in a refrigerator at 4 ºC. More diluted solutions were prepared by several dilutions with water.

Solution of a 1×10^{-3} mol L^{-1} prednisolone solution was prepared daily by dissolving 0.0402 g prednisolone acetate (99 %) in propanol-water (50:50, v/v) and diluted to the mark in a 100 mL volumetric flask. The solution was kept in a refrigerator at 4 ºC and in dark. More dilute solutions were prepared by serial dilution with water. Britton-Robinson buffer (0.1 mol L^{-1}) in the pH range of 2-9 was used, throughout.

UV-Vis absorption spectra were measured on an UV-vis spectrophotometer, Perkin-Elmer (Lambda 25), with quartz cells of 1 cm.

A pentium IV (2.53 MHz) computer controlled all of the setting and data processing. pH of the solution was checked by a pH meter (Metrohm, Model 691) with a double junction glass electrode.

Preparation of real samples: Serum/or whole blood samples were obtained and stored frozen until the assay. Into each of 10 centrifugation tubes containing a certain concentration of sulfacetamide and/or prednisolone, 0.10 mL of human serum/or whole blood sample was transferred and then mixed well with 1 mL of methanol to precipitate blood proteins. The precipitated proteins were separated by centrifuging of the mixture for 20 min at 4000 rpm. The clear supernatant layer was filtered through a 0.45 μm milli-pore filter to produce protein-free serum/or whole blood. The protein-free sample was transferred into a 25 mL calibrated flask, completed to the volume with Britton-Robinson buffer (pH 3; 0.1 mol L^{-1}) and then introduced to the optical cell.

For preparation of urine samples, 1 mL of urine was acidified with about 50 μL of concentrated HCl and extracted for 2 min with 5 mL of diethyl ether in a 16 × 150 mm screw-capped tube by shaking rapidly on a wrist-action shaker and then centrifuged. 4 mL aliquot of the ether phase was removed and re-extracted for the same time interval with 5 mL of diethyl ether, followed by centrifugation and the ether was then removed[35]. The residual was diluted in a 100 mL volumetric flask.

General procedure: An aliquot of the solution containing sulfacetamide and/or prednisolone and 1 mL Britton-Robinson buffer solution (pH 3; 0.1 mol L^{-1}) was added into a 10 mL of volumetric flask and diluted to the mark with water. The solution was then allowed to stand for 10 min at room temperature. After that a portion of the solution was transferred into a quartz cell to measure its absorbance at appropriate wavelength pairs (230.6 and 268.4 nm) for determination of sulfacetamide and (246.3 and 294.7 nm) for determination of prednisolone. The concentration range of sulfacetamide and prednisolone for construction of HPSAM curves was 10-100 μmol L^{-1} for both species.

Theoretical background: Consider a binary mixture of sulfacetamide and prednisolone compounds with typical spectra shown in Fig. 1. In the absence of matrix effect the measured absorbance of the sample at each wavelength is the sum of the individual absorbances of the sulfacetamide and prednisolone species at this wavelength:

$$A_i = \varepsilon_{i,s} c_s^0 + \varepsilon_{i,p} c_p^0 + A_i^0 \quad (1)$$

where A_i = absorbance of the mixture at wavelength λ_i, c_s^0 and c_p^0 are the concentrations of sulfacetamide and prednisolone in the sample, $\varepsilon_{i,s}$ and $\varepsilon_{i,p}$ are the molar absortivity coefficients at wavelength λ_i for corresponding compounds and A_i^0 is the residual absorbance at λ_i, which can be nearly omitted by subtracting the absorbance of blank. Therefore eqn. 1 can be written as:

$$A_i = A^0_{i,s} + A^0_{i,p} \quad (2)$$

$A^0_{i,s}$ and $A^0_{i,p}$ are the individual absorbances of the sulfacetamide and prednisolone compounds in the considered sample. According to the HPSAM basis the quantification

of each analyte (X) in the presence of the other as an interferent (Y), can be performed by the construction of two standard addition plots for the analyte, with $M_{X,1}$ and $M_{X,2}$ slopes, at two previously selected wavelengths, λ_1 and λ_2, which intersect at the H-point with $[-C_{H(X)}, A_{H(Y)}]$ coordinates. For example if sulfacetamide is considered as analyte, the H-point depends on its concentration c_s^0:

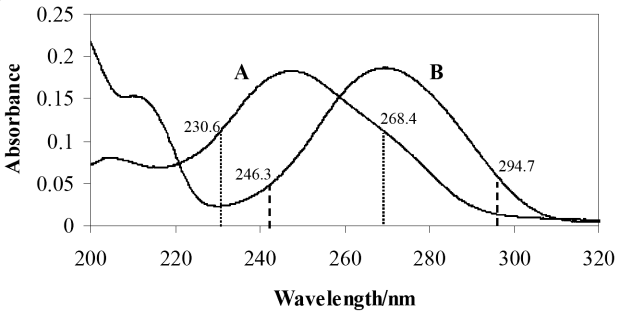

Fig. 1. Absorption spectra of (a) 10 μmol L^{-1} prednisolone and (b) 10 μmol L^{-1} sulfacetamide at pH 3. The dashed lines are selected wavelengths for applying HPSAM

$$-c_{H(s)} = \frac{(A^0_{s,1} - A^0_{s,2}) + (A^0_{p,1} - A^0_{p,2})}{(M_{s,1} - M_{s,2})} \quad (3)$$

$$= -c^0_s + \frac{(A^0_{p,1} - A^0_{p,2})}{(M_{s,1} - M_{s,2})} \quad (4)$$

By selecting wavelengths λ_1 and λ_2 in such a away that the prednisolone absorbance were equal

$$A_{p,1} = A_{p,2} \quad (5)$$

Then the abscissa of the H-point will be the sulfacetamide concentration in the sample, c_s^0. The concentration of prednisolone can be determined according to conventional HPSAM from the correlation between A_H and prednisolone concentration.

H-point standard addition method (HPSAM) based on simultaneous standard addition of the two species as reported by Campins-Falco *et al.*[34] permits one to obtain concentration of both analyte in the sample from a unique calibration set. The required data to apply the method are the response of the sample and the response of the sample spiked with known amounts of both analytes at previously selected variables.

For simultaneous determination of sulfacetamide and prednisolone by the proposed method, let us suppose that λ_1 and λ_2 were selected wavelengths according to eqn. 5. By representing the analytical signal, absorbance at two previously selected wavelengths λ_1 and λ_2 *versus* the concentration added of sulfacetamide, two lines would be obtained with intercepts $(A^0_{s,1} + A^0_{p,1})$ and $(A^0_{s,2} + A^0_{p,2})$ and slopes:

Slope at λ_1: $M_{s,1} + (c^i_p/c_s^i)M_{p,1}$ $i = 0, 1, \ldots, n$ (6)

Slope at λ_2: $M_{s,2} + (c^i_p/c_s^i)M_{p,2}$ $i = 0, 1, \ldots, n$ (7)

$M_{s,1}$, $M_{p,1}$, $M_{s,2}$ and $M_{p,2}$ are the slopes due to the addition of sulfacetamide and prednisolone in the lines obtained at λ_1 and λ_2; c_s^i and c_p^i are the concentrations of considered pharmaceutical compounds added in the i solution; n is the number of additions. When i = 0 corresponds to the solution where only the sample exists. Both calibration lines intersect at the H-point, with coordinates [$-C_{H(s)}$, $A_{H(p)}$], where $-C_{H(s)}$ is the unbiased concentration of sulfacetamide concentration. In this case the abscissa of the H-point will be:

$$-c_{H(s)} = \frac{(^0_{s,1} - A^0_{s,2}) + (A^0_{p,1} - A^0_{p,2})}{(M_{s,2} - M_{s,1}) + (c_p^i / c_s^i)(M_{p,2} - M_{p,1})} = \frac{(A^0_{s,1} - A^0_{s,2})}{(M_{s,2} - M_{s,1})} \quad (8)$$

The incorrigible error due to the presence of prednisolone has transformed into a constant systematic error as the HPSAM basis predicts. It can be proved that $A_{H(p)}$ is equivalent to:

$$A_{H(p)} = A^0_{p,1} - (c_p^i/c_s^i)M_{p,1}c_{H(s)} = A^0_{p,2} - (c_p^i/c_s^i)M_{p,2}c_{H(s)} \quad (9)$$

In the same way as for sulfacetamide, by selecting two wavelengths λ_3 and λ_4 in such a way that sulfacetamide present the same absorbance ($A_{s,3} = A_{s,4}$), similar expressions can be obtained for prednisolone. The analogous expressions for eqns. 8 and 9 in the analysis of prednisolone are:

$$-c_{H(p)} = \frac{(A^0_{p,3} - A^0_{p,4}) + (A^0_{s,3} - A^0_{s,4})}{(M_{p,4} - M_{p,3}) + (c_p^i / c_s^i)(M_{s,4} - M_{s,3})} = \frac{(A^0_{p,3} - A^0_{p,4})}{(M_{p,4} - M_{p,3})} \quad (10)$$

and $$A_{H(s)} = A^0_{s,3} - (c_p^i/c_s^i)M_{s,3}c_{H(p)} = A^0_{s,4} - (c_p^i/c_s^i)M_{s,4}c_{H(p)} \quad (11)$$

The precision of the obtained results will depend on the value of the denominator in eqns. 8 and 10. The higher value of the denominator causes the higher in precision of the results. In addition, the precision of the results will also depend on the relation of and the sample composition. Both variables studied in results and discussion section.

RESULTS AND DISCUSSION

The absorption spectra of sulfacetamide and prednisolone under certain experimental conditions have shown in Fig. 1. As can be seen, the maximum wavelengths of two compounds are very close to each other and their spectra are highly overlapped. Therefore, determination of two pharmaceutical compounds in the presence of each other is impossible by classical spectrophotometry. Therefore, we used HPSAM with simultaneous additions of both analytes for resolving of additive interference and matrix effect simultaneously.

Effect of operational parameters: In order to optimize the procedure for the simultaneous determination of sulfacetamide and prednisolone, we studied the effect of pH on the sensitivity and selectivity of two species separately. As it has shown in Fig. 2, there weren't significant changes in the absorbance spectra of sulfacetamide and prednisolone in the pH range 2-10, But, at pH < 6 the overlap between two spectra decreased. Therefore pH of 3 was selected for obtaining higher selectivity.

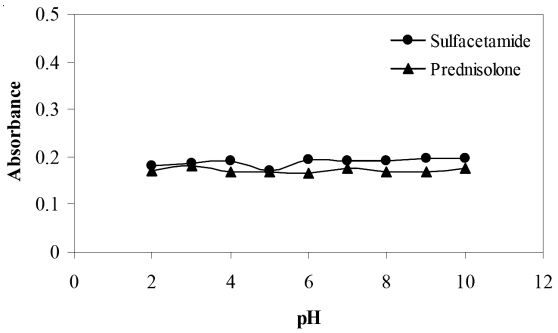

Fig. 2. Effect of pH on the maximum absorption spectra of sulfacetamide and prednisolone. Conditions: Britton Robinson buffer (0.1 mol L^{-1}), sulfacetamide concentration 10 μmol L^{-1}, prednisolone concentration 10 μmol L^{-1}

Wavelength selection: To select the appropriate wavelength pairs for using HPSAM the following principles should be applied. At these selected wavelengths, the analyte signals must be linear with concentrations and the interferent signal must be remains unchanged by changing the analyte concentration. Also the analytical signal for a mixture containing the analyte and the interfering should be equal to the sum of the individual signals of the two components. In addition, the difference in the slopes of the two straight lines measured at two selected wavelengths must be as large as possible in order to get good accuracy and sensitivity[31].

Consequently, for determination of sulfacetamide and prednisolone, we selected two pairs of wavelengths on the sulfacetamide and prednisolone spectra. In this case there were several pairs of wavelengths. As it observed from Fig. 1, the best wavelength pairs were 230.6-268.4 and 246.3-294.7 nm for determination of sulfacetamide and prednisolone, respectively. Standard solutions of two species were initially tested to validate the applicability of the chosen wavelengths. Fig. 3 is a typical H-point standard addition calibration lines constructed at selected wavelengths.

The effect of the concentration ratio of sulfacetamide to prednisolone on the accuracy of the method was also studied. From the obtained results it was concluded that the best results would be obtained when the concentration ratio of sulfacetamide to prednisolone in standard addition was 1:1 (mol/mol). Therefore, the concentration ratio of 1:1 was selected for further studies.

Accuracy: Several synthetic samples with different concentration ratios of sulfacetamide to prednisolone were analyzed by using the suggested method (Table-1). The accuracy of the results is satisfactory at the concentration ratios of 1:6-10:1 sulfacetamide to prednisolone.

Reproducibility of the method: To check the reproducibility of the method, standard deviations of calculated concentrations of sulfacetamide and prednisolone by the proposed method were determined. As it is shown in Table-2, the proposed method has a good reproducibility for simultaneous determination of sulfacetamide and prednisolone.

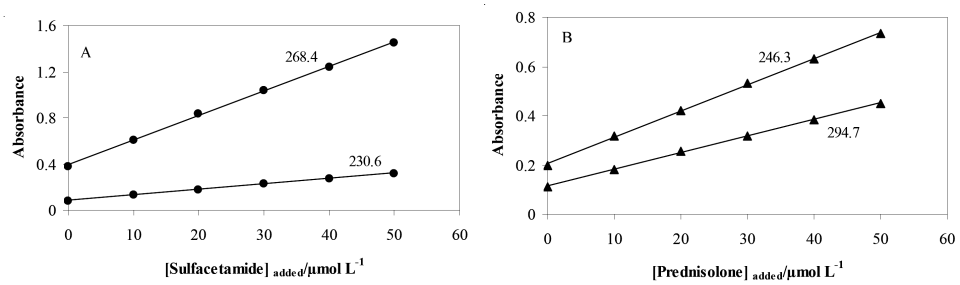

Fig. 3. H-Point standard addition plots with simultaneous additions of both analytes for determination of a mixture of sulfacetamide (10 µmol L^{-1}) and prednisolone (10 µmol L^{-1}). (A) H-point standard addition plots at wavelengths 230.6 and 268.4 nm (B) H-point standard addition plots at wavelengths 246.3 and 294.7 nm at pH 3

TABLE-1
DETERMINATION OF SULFACETAMIDE AND PREDNISOLONE IN SOME SYNTHETIC MIXTURES

A-C Equation	R^2	C_{added} (µmol L^{-1})		C_{found} (µmol L^{-1})	
		SFA	P	SFA	P
A268.4 = 0.0300C + 0.2970	0.9992	10	10	10.34	10.34
A230.6 = 0.0119C + 0.1098	0.9949				
A246.3 = 0.0219C + 0.2133	0.9996				
A294.7 = 0.0081C + 0.0706	0.9991				
A268.4 = 0.0213C + 0.3933	0.9992	20	20	18.57	22.95
A230.6 = 0.0047C + 0.0850	0.9985				
A246.3 = 0.0107C + 0.2063	0.9993				
A294.7 = 0.0067C + 0.1145	0.9992				
A268.4 = 0.0302C + 1.3937	0.9995	20	100	20.19	94.39
A230.6 = 0.0113C + 1.0120	0.9848				
A246.3 = 0.0233C + 1.5493	0.9937				
A294.7 = 0.0092C + 0.2183	0.9988				
A268.4 = 0.0259C + 1.0408	0.9998	60	10	57.99	9.65
A230.6 = 0.0112C + 0.1883	0.9999				
A246.3 = 0.0200C + 0.4636	0.9998				
A294.7 = 0.0076C + 0.3439	0.9987				
A268.4 = 0.0252C + 0.7431	0.9999	10	60	8.50	63.59
A230.6 = 0.0113C + 0.6250	0.9997				
A246.3 = 0.0202C + 0.9660	0.9978				
A294.7 = 0.0067C + 0.1075	0.9998				
A268.4 = 0.0280C + 2.0270	0.9893	100	10	102.87	9.22
A230.6 = 0.0110C + 0.2782	0.9938				
A246.3 = 0.0034C + 0.7497	0.9845				
A294.7 = 0.0170C + 0.8479	0.9993				

Interferents: An attractive feature of an analytical procedure is its relative freedom from interferences. The selectivity of the proposed procedure for the assay of sulfacetamide and prednisolone was identified by studying the effect of excipients

TABLE-2
REPLICATE MEASUREMENTS OF SULFACETAMIDE AND PREDNISOLONE IN SOME SYNTHETIC MIXTURES

C_{added} (μmol L^{-1})		C_{found} (μmol L^{-1})	
HCT	FU	HCT	FU
10	10	10.07	10.94
10	10	10.61	10.82
10	10	10.46	11.15
10	10	10.55	10.80
10	10	10.26	10.51
10	10	10.04	11.86
Average		10.33	11.01
standard deviation		0.24	0.46
10	50	10.01	53.06
10	50	10.11	51.19
10	50	10.16	53.67
10	50	10.14	51.78
10	50	10.60	51.24
10	50	9.67	50.09
Average		10.11	51.84
standard deviation		0.29	1.31
50	10	52.86	9.45
50	10	53.13	9.53
50	10	50.67	9.54
50	10	49.50	10.06
50	10	50.21	9.31
50	10	50.95	10.28
Average		51.22	9.69
standard deviation		1.46	0.38

that often accompany with sulfacetamide and prednisolone in pharmaceutical formulations and biological fluids. Therefore, samples containing 10 μmol L^{-1} sulfacetamide and/or prednisolone in the absence and presence of excipients were analyzed by means of the proposed procedure. Among all of the excipients, rifampin, warfarin, anticoagulants and estrogens may interact with prednisolone in the combination dosage. Therefore their interfering affects did not study in this section. For other constituents, tolerance limit was defined as the concentrations which gave an error of ≤ 5 % in the determination of pharmaceutical compounds. The effects of all examined compounds at several molar ratios over sulfacetamide and prednisolone on the measured analytical concentrations are given in Table-3. The results show no significant interference from excipients.

Application: In addition, for showing the applicability of the method, some biological fluids with complex matrixes were spiked with sulfacetamide and prednisolone and proposed method was applied for simultaneous determination of the species. The results showed (Table-4) that the proposed method can be applied for analysis of appropriate compounds in real samples with satisfactory results.

Employing the proposed procedure on several synthetic samples showed that, C_H (concentration of analyte) was independent of the concentration of interference. Limit of detection was calculated as $3S_{CH}$, where S_{CH} is standard deviation of several (n = 6) replicated measurements of zero concentration of analyte by the HPSAM. The corresponding values obtained for sulfacetamide and prednisolone were 0.41 and 0.77 μmol L^{-1}, respectively.

TABLE-3
MAXIMUM TOLERABLE CONCENTRATION OF INTERFERING SPECIES WITH SULFACETAMIDE (10 μmol L^{-1}) AND PREDNISOLONE (10 μmol L^{-1})

Species	Tolerance concentration (μmol L^{-1})
Fe^{2+}, Fe^{3+}, Cu^{2+}, NO_3^-, I^-, K^+, Na^+, CH_3COO^-, C_2H_5OH, CH_3OH	10000
Mg^{2+}, SO_4^{2-}, Al^{3+}, Cl^-, CO_3^{2-}, Cd^{2+}, PO_4^{3-}, Ca^{2+}, Zn^{2+}, Br^-, Co^{2+}, Mn^{2+}, C_6H_5COONa	1000
Vitamin B_1, vitamin B_6, vitamin C, folic acid, glucose	100

TABLE-4
DETERMINATION OF SULFACETAMIDE AND PREDNISOLONE IN SOME BIOLOGICAL FLUIDS

Sample	C_{added} (μmol L^{-1})		C_{found} (μmol L^{-1})		Recovery	
	SFA	P	SFA	P	SFA	P
Whole blood	–	–	< DL*	< DL	–	–
	10.0	30.0	10.04 ± 0.23**	30.68 ± 1.14	100.4	102.2
	50.0	50.0	50.77 ± 2.5	50.21 ± 1.9	101.5	100.4
Plasma	–	–	< DL	< DL	–	–
	10.0	10.0	9.96 ± 0.51	10.26 ± 0.13	99.6	102.6
	20.0	20.0	20.21 ± 1.09	20.51 ± 1.35	101.0	102.5
Urine	–	–	< DL	< DL	–	–
	20.0	20.0	20.24 ± 0.35	20.24 ± 0.52	101.2	101.2
	40.0	40.0	40.53 ± 0.09	40.35 ± 0.52	101.3	100.8

*Detection limit, **± Confidence interval for 3 replicate determinations.

Conclusion

It has demonstrated that the HPSAM with simultaneous addition of both analytes, permits the quantification of sulfacetamide and prednisolone in a binary mixture monitored by UV/vis spectrophotometry. Both analytes can be analyzed free from multiplicative (matrix effect) and additive (interferent) errors at only one standard addition procedure. The proposed method provides satisfactory results in synthetic and real mixtures. According to this study, HPSAM with simultaneous addition of both analytes is recommended as a very suitable choice to resolve accurately overlapped UV/vis spectra of pharmaceutical compounds.

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