

Spectrofluorimetric Estimation of Some Sulfhydryl – Containing Drugs by Demasking Reaction of the Palladium Chelate of 8-Hydroxyquinoline-5-Sulfonic Acid

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Abstract

A simple and sensitive spectrofluorimetric method has been developed for the determination of some selected sulfhydryl-containing drugs namely Acetylcysteine (ACS), Captopril (CAP) and Mesna (MSN).

The method is based on the interaction of the drugs with potassium (5-sulfoxino) palladium II in alkaline medium in presence of magnesium ions, where the sulfhydryl group combines with palladium from the non-fluorescent potassium bis (5-sulfoxino) palladium II. The resulting 8-hydroxy-5-quinoline sulfonic acid coordinates with magnesium to form the fluorescent chelate that is a measure of the amount of sulfhydryl containing drug analyzed. The fluorescence intensity was measured at an emission wavelength of 485 nm, by excitation at 345 nm. All the experimental parameters affecting the reaction were studied and optimized. The proposed method was applicable over the concentration range of 0.04-0.44 µg/mL for the three drugs and was applied for their determination in bulk form and in pharmaceutical preparations without interference from common excipients. The assay results were statistically compared with those obtained from previously reported methods where no significant difference was found between them. The selectivity and the stability-indicating aspect of the proposed method were confirmed by preparing the disulphides of the studied drugs and applying the reaction to the parent drugs in presence of their disulphides where no interference was detected from these related substances. By virtue of its high sensitivity, the proposed method was also extended to analyze the drugs in spiked human plasma and urine.

Keywords: Sulfhydryl-containing drugs; Potassium (5-sulfoxino) palladium II; Spectrofluorimetry; Plasma; Urine

Introduction

Mesna (MSN) is chemically known as sodium 2-sulfanylethanesulfonate [1]. It is used for the prevention of urothelial toxicity in patients being treated with the antineoplastics ifosfamide or cyclophosphamide [2]. MSN is official in both BP 2013 [1] and USP 34 [3], both describe an indirect iodimetric titration method for the determination of the drug in its pure powder form. Screening the literature revealed that various techniques were used for MSN quantitation. Of these we can mention: A kinetic spectrofluorimetric method using cerium (IV) as an oxidizing agent [4], different spectrophotometric methods utilizing several reagents such as potassium permanganate [5], methyl orange and congo red [6], N,N-dimethyl-p-phenylenediamine [7] and ferric solution [8]. Other techniques include High Performance Liquid Chromatography (HPLC) [9,10], capillary electrophoresis (CE) [10] and Raman spectroscopy [11]. In biological fluids, most of the methods published for MSN quantitation were HPLC methods [12-14].

Acetylcysteine (ACT) is chemically known as (2R)-2-(Acetylamino)-3-sulfanylpropanoic acid [1]. It is used as a mucolytic agent for treatment of respiratory disorders associated with productive cough [2]. ACT is official in both BP 2013 [1] and USP 34 [3]. The BP

describes a titrimetric method for the determination of the drug in bulk form, a liquid chromatographic method for its determination in eye drops and a titrimetric method for its determination in injections. The USP describes a liquid chromatographic method for the analysis of ACT powder and solution. By reviewing the scientific literature, we found that ACT has been determined in many reports either in single form or in combination with other drugs. Concerning spectrofluorimetric technique, to the best of our knowledge only one report was introduced for ACT determination, it is based on the quenching effect of the drug on the red fluorescence of oligonucleotide-protected silver nanoclusters (Ag NCs) [15]. Many spectrophotometric methods were used for ACT estimation in bulk form and in pharmaceuticals; some of these methods were based on redox reactions due to the reducing effect that ACT exhibits, of these we can mention the oxidation of ACT with Fe³⁺ followed by reaction of the produced Fe²⁺ with 2,4,6-tripyridyl-s-triazine (TPTZ) [16]. Another method relies on the reduction of Cu (II) neocuproine to Cu (I) neocuproine in Britton-Robinson buffer [17]. Other published methods depend on derivatization of ACT with various reagents such as 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) [18], 2,4-dinitrofluorobenzene (DNFB) [19] and a new reagent called ethylpropiolate (EPL) which was used for on-line derivatization of ACT, where the formed derivative was detected by using sequential injection analysis [20]. Flow injection spectrophotometric procedures have been proposed for ACT estimation. These procedures involve the reaction of ACT by different

means such as: reaction of thiol group with o-phthalaldehyde and isoleucine [21], complexation reaction of the drug with Pd²⁺ [22] and a redox reaction employing generation of prussian blue product [23]. Chromatographic methods were also applied for the analysis of this compound. These include HPLC methods with different detection systems such as: UV detector [24,25]; fluorescence detector after pre-column derivatization with monobromobimane (Thiolyte MB) [26] or aroylacrylic acids [27]. Furthermore, CE has been one of the chromatographic techniques that was successfully applied to the analysis of ACT [28]. In biological fluids, HPLC has been the most predominant technique for the quantification of ACT and fluorescence detection was applied by using different reagents such as N-(1-pyrenyl) maleimide [29] or ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F) [30] as precolumn fluorescence labeling reagents while ortho-phthalaldehyde was used for post column derivatization [31]. Moreover, liquid chromatography tandem mass spectrometry (LC-MS/MS) was a powerful tool applied for the estimation of ACT in plasma and urine [32,33].

Captopril (CAP) is a sulfhydryl-containing inhibitor of ACE which converts angiotensin I to angiotensin II (a potent endogenous vasoconstrictor substance). It is used in the management of hypertension, in heart failure, after myocardial infarction and in diabetic nephropathy. The drug has free radical scavenging ability attributable to its thiol group. This group has been suggested to be cardio protective and to contribute to the ability of CAP to scavenge free radicals following myocardial ischemia, thereby reducing the reperfusion injury [2].

Captopril (CAP) is official in BP 2010 [1] and USP 34 [3], both pharmacopoeias describe HPLC procedures for its analysis. The analytical profile of the drug with several references describing its determination in pharmaceutical preparations and in biological fluids up to 1982 has been reviewed [34]. Different spectrophotometric procedures have been reported for the determination of CAP; these include reaction with promethazine [35], palladium II chloride in Britton-Robinson buffer [36], 2,4-dinitrofluorobenzene in borate buffer [37], Ellman reagent [38], DTNB [39], chloramines-T [40], chloranilic acid [41], iodate [42], copper II-neocuproine [43], 1,10-phenanthroline [44], potassium ferricyanide [45], carbon disulfide in alkaline medium [46], partial least squares spectrophotometry [47], kinetic spectrophotometry [48], derivative spectrophotometry [49]. Captopril has been assayed spectrofluorimetrically either after reaction with different organic modifiers [50,51] or after reduction of Ce (IV) to fluorescent Ce (III) [52,53].

The electrochemical characteristics of CAP were determined by d.c., differential pulse polarography and voltammetry [54-59]. The drug has been also assayed by potentiometry [60,61] and anodic polarography [62]. Also, conductimetric titration [63] and atomic absorption spectrophotometry after complexation with palladium [64] or chelation of its trithiocarbamate derivative with divalent metals [46] have been applied for its determination. The drug and its degradation products have been determined by capillary electrophoresis [65].

Other techniques include HPLC [66-68] and indirect determination using sodium nitrate and thiocyanate by flotation separation [69].

In biological fluids CAP has been determined by gas chromatography [70-72], kinetic spectrophotometry [73], HPLC [74-77] and HPLC with fluorimetric detection after pre [78,79] or post [80] column derivatization.

In the present work, a simple and sensitive spectrofluorimetric method has been developed for the determination of the three selected sulfhydryl-containing drugs. The method is based on demasking reaction of palladium chelates. These demasking reactions were formerly applied for the estimation of cyanide and sulfide [81,82].

The fluorimetric detection of cyanide by demasking reactions has been reported by Feigl and Feigl [83] and by Feigl and Heisig by the demasking of oxine (8-quinolinol) from copper (II) oxinate, which permits aluminum ion present to form the fluorescent aluminum (III) oxinate. In the developed method, the selected drugs interact with potassium (5-sulfoxino) palladium II in alkaline medium in presence of magnesium ions, where the sulfhydryl group combines with palladium from the non-fluorescent potassium bis (5- sulfoxino) palladium II. The resulting 8-hydroxy-5-quinoline sulfonic acid coordinates with magnesium to form the fluorescent chelate. The fluorescent chelate is a measure of the amount of sulfhydryl containing drug analyzed.

The selectivity and the stability-indicating aspect of the proposed method were confirmed by applying the reaction to the parent drugs in presence of their disulphides, where no interference was detected from these related substances.

Although the literature included only very few spectrofluorimetric procedures for the assay of MSN, ACT and CAP, there was still a need to make some improvements to the previously published methods, in order to achieve better selectivity and analytical performance. Therefore, our objective was to develop a simple, rapid, specific and sensitive method that could be applied to the analysis of the three drugs in their pharmaceutical preparations, in biological fluids or even to study their pharmacokinetics in future work.

Methodology

Instrumentation

A Shimadzu (Kyoto, Japan) model RF-1501 Version 3.0 spectrofluorophotometer equipped with 1-cm quartz cell and a 150 W Xenon lamp.

Centrifuge PLC series-Model: PLC-03, Power: 220 V/50 Hz; 0.65 A. Serial No.: 909689. Gemmy Industrial Crop. Made in Taiwan, Associated with Cantic, Inc., USA.

A thermostatically controlled water bath, Kottermann, Germany.

Digital pH meter 3310 Jenway.

Materials and reagents

Acetylcysteine and captopril were kindly provided by EEPI and Pharco Pharmaceuticals and were used without any further purification.

Mesna was purchased from Fluka-Germany.

All solvents and chemicals were of analytical grade.

Palladium chelate was synthesized [81] in the lab.

Urine and plasma samples were donated from healthy volunteers.

Glycine buffer was prepared by dissolving 5 g glycine in 100 mL purified water, 0.1 N NaOH was added till pH 11 then the volume was completed to 1 L using purified water.

Palladium chelate solution was prepared by dissolving 50 mg of potassium (5-sulfoxino) palladium II in 100 mL purified water followed by dilution of 25 mL to 100 mL using purified water to obtain a final concentration of 125 µg/mL.

Magnesium chloride solution was prepared by dissolving 1.2 g of magnesium chloride hexahydrate in 100 mL glycine buffer.

Peroxyacetic acid solution was prepared by diluting 7 mL of 30 v/v hydrogen peroxide to 100 mL with glacial acetic acid, the solution was heated in water bath at 70°C for one hour. This solution was used to prepare the disulfides.

Trichloroacetic acid solution (TCAA) was prepared by dissolving 10 g into 100 mL purified water.

Preparation of the stock solutions

Stock solutions of the drugs were prepared in purified water, separately, and diluted to obtain a final concentration of 4 µg /mL for each.

General procedure

Construction of calibration curves: Aliquots of (0.1-1.1) mL in 0.2 mL increments of the stock solution of each drug were transferred to 10 mL volumetric flasks, treated with 1 mL of 125 µg/mL palladium chelate solution followed by 2 mL of 1.2 g% magnesium chloride solution and the volumes were completed to 10 mL using glycine buffer pH 11.

The contents of the flasks were mixed and kept to stand for 15 min at room temperature, then, the relative fluorescence intensities of the prepared solutions were measured at an emission wavelength of 485 nm, with excitation at 345 nm against a similarly prepared blank.

Application to pharmaceutical preparations

Capoten tablets assay (25 mg Captopri/tablet): Twenty tablets were accurately weighed and ground to a fine powder, then an amount of the mixed tablet powder equivalent to 25 mg CAP was accurately weighed and transferred to a 100 mL volumetric flask, extracted using 30 mL purified water, sonicated for 20 min, and the volume was completed to 100 mL with the same solvent. A 0.8 mL aliquot was diluted to 50 mL with purified water, and the procedure was completed as described under "construction of calibration curves" in section 2.4.1.

ACC effervescent tablets assay (200 mg Acetylcysteine/tablet): Twenty tablets were accurately weighed and ground to a fine powder, then an amount of the mixed tablet powder equivalent to 200 mg ACS was accurately weighed and transferred to a 100-mL volumetric flask, extracted using 30 mL purified water, sonicated for 20 min, and the volume was completed to 100 mL with the same solvent. A 2 mL aliquot was diluted to 50 mL with water followed by further dilution of 2.5 mL to 50 mL with purified water, and the procedure was completed as described under "construction of calibration curves" in section 2.4.1.

ACC sachets (200 mg Acetylcysteine/sachet): The contents of twenty sachets were mixed, an amount equivalent to 200 mg ACS was accurately weighed and transferred to 100-mL volumetric flask, extracted using 30 mL purified water, sonicated for 20 min and the volume was completed to 100 mL with the same solvent. A 2 mL aliquot was diluted to 50 mL with water followed by further dilution of 2.5 mL to 50 mL with purified water, and the procedure was completed as described under "construction of calibration curves" in section 2.4.1.

Uromitexan ampoule assay (400 mg mesna/4 mL ampoule): The content of one ampoule was accurately diluted to 100 mL with purified water in a 100-mL volumetric flask. A 1 mL aliquot of this solution was diluted to 50 mL with water, followed by further dilution of 2.5 mL to 50 mL with purified water, and the procedure was completed as described under "construction of calibration curves" in section 2.4.1.

Application to biological fluids

Application to plasma and urine samples spiked with the three drugs: Different aliquots from standard solutions of acetylcysteine, captopril and mesna were transferred into separate centrifugal tubes containing 4 mL plasma or urine. The tubes were shaken for 2 min using vortex tube shaker.

The solutions were deproteinized twice each with 1 mL trichloroacetic acid (TCAA) and centrifuged for 30 min at 8000 rpm. The centrifugates were transferred to clean centrifuge tubes and the residues were washed twice each with 1 mL of TCAA. The washing liquids were added to the centrifugates and centrifugation was repeated for 10 min. The clear centrifugates were filtered through 0.45 µm filter and the volumes were adjusted to 10 mL with purified water.

A 1 mL aliquot from each drug test solution was transferred to 10-mL volumetric flask (in case of plasma) and 100 mL volumetric flask (in case of urine), neutralized with drops of saturated sodium carbonate solution till no effervescence.

The reagents were added as described under" construction of calibration curves", and then the volumes were completed to 10 mL (in case of plasma) and to 100 mL (in case of urine), with glycine buffer. The solutions were left to stand at room temperature for 15 min. The relative fluorescence intensities were measured using the above mentioned fluorescence parameters, against a similarly treated blank.

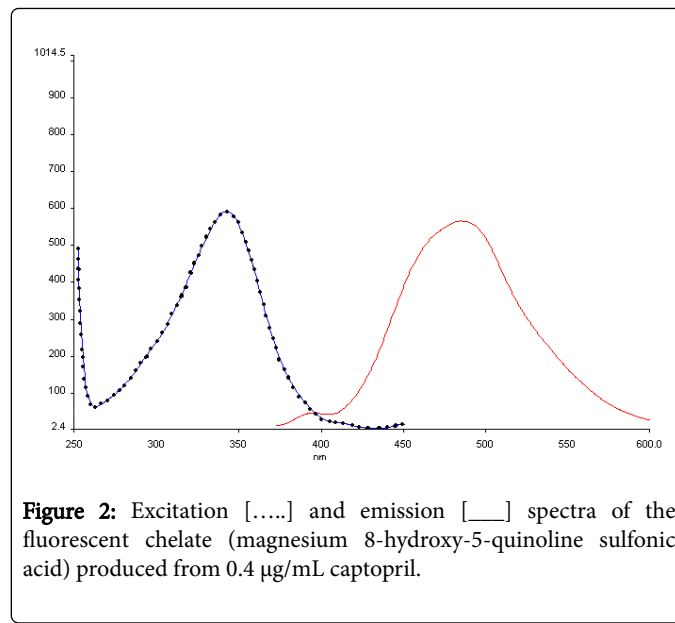
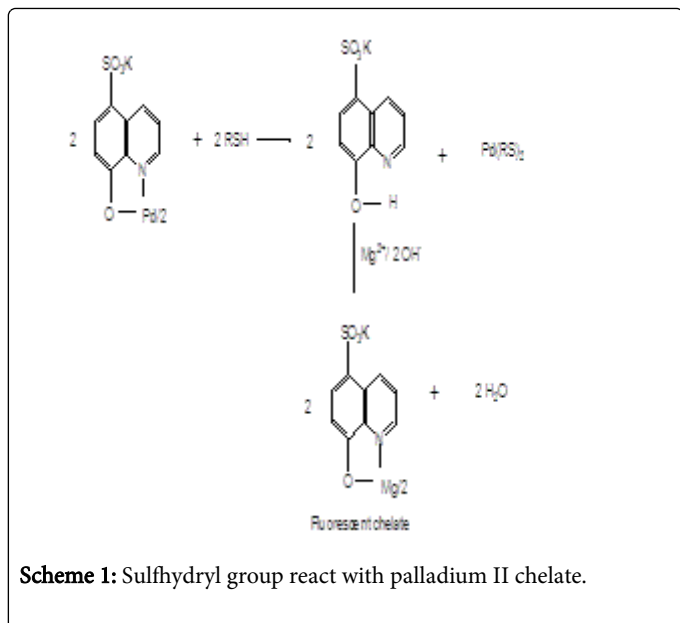
Results and Discussions

Drugs containing a sulfhydryl group react with palladium II chelate of 8-hydroxy-5-quinoline sulfonic acid to liberate 8-hydroxy-5-quinoline sulfonic acid moiety, which in turn coordinates with magnesium ions to form a florescent chelate. The proposed reaction mechanism is shown in the Scheme 1.

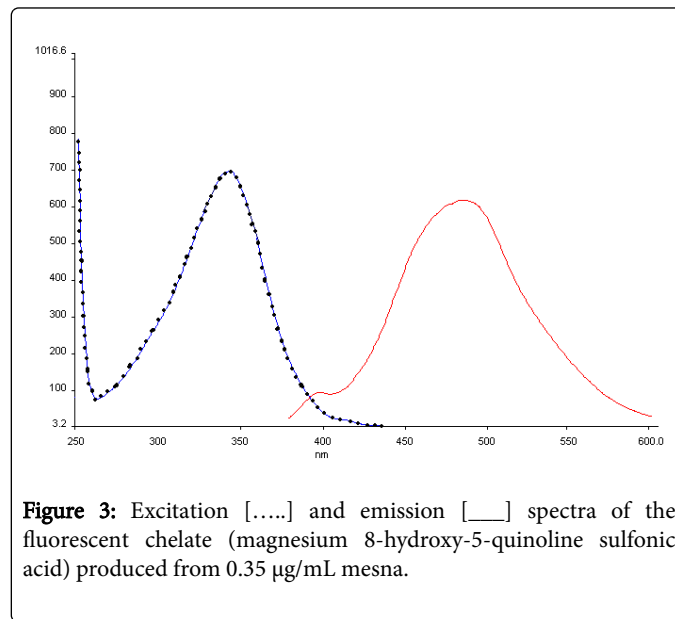
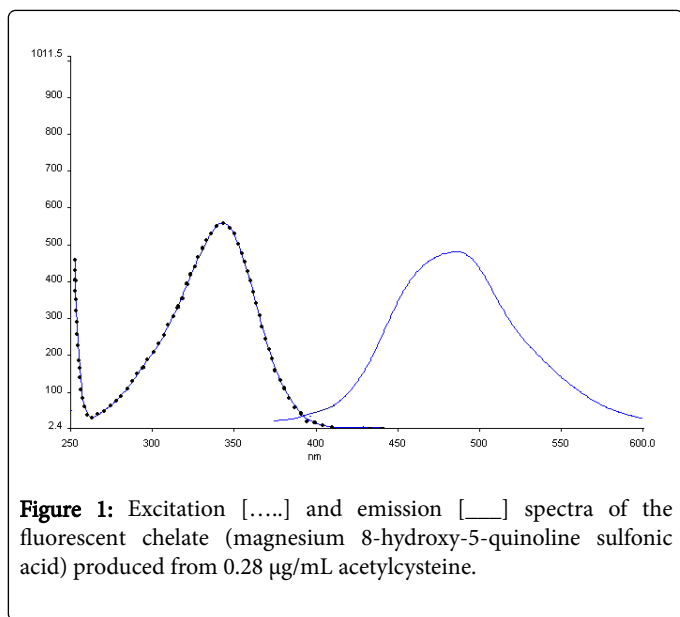
Optimization of experimental factors affecting fluorescent chelate formation

Effect of palladium chelate concentration: The effect of palladium chelate concentration was studied by varying its volume from 0.1-3.5 mL (from the reagent solution prepared as 125 µg/mL) in 0.5 mL increments while keeping all other parameters constant.

It was found that using small concentrations corresponding to volumes below 0.5 mL resulted in a poor formation of the fluorescent magnesium chelate, while higher concentrations corresponding to volumes above 1 mL resulted in quenching of the florescence; therefore 0.5-1 mL was the optimum volume of the palladium chelate solution for the three investigated drugs. Actually 0.6 mL was chosen for the three drugs as the optimum volume.



The excitation and the emission spectra of the magnesium chelates of the investigated drugs are shown in Figures 1-4.



Effect of magnesium chloride concentration: The effect of magnesium chloride concentration was investigated by trying different volumes of 1.2 % solution ranging from 0.2-4 mL.

It was found that low concentrations corresponding to volumes below 2 mL resulted in poor formation of the fluorescent chelate, while high concentrations corresponding to volumes above 2 mL resulted in a plateau; therefore 3 mL was chosen as the optimum volume (Figure 5).

Effect of buffer type: The use of phosphate buffer resulted in the precipitation of Mg^{2+} ions in the form of the insoluble magnesium phosphate; therefore, glycine was the buffer of choice.

Effect of buffer pH: Glycine buffer of different pH values was tried and it was found that the emission peak was shifted to a lower wavelength 400 nm instead of 485 nm at pH 9, the peak was shifted, distorted and of lower intensity at pH 8 and became even more distorted at pH 7.

On the other hand, the peak shape, intensity and emission wavelength did not change at pH 10, 11 and 12. So pH 11 was chosen for this study.

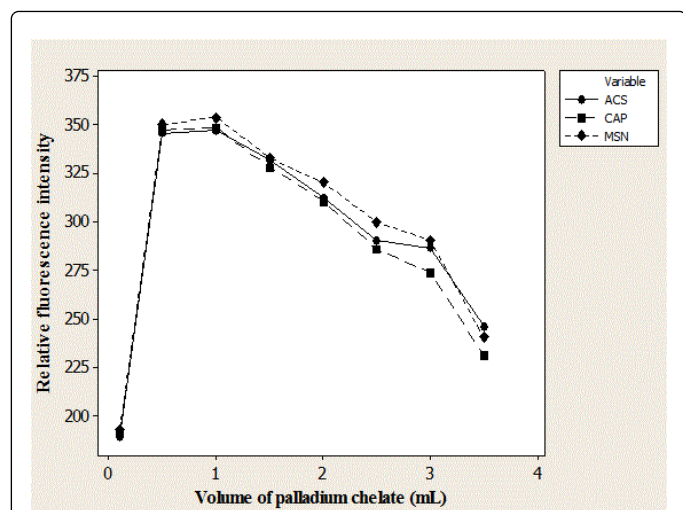


Figure 4: Effect of the volume of palladium chelate solution on the fluorescence intensity produced from ACS, CAP and MSN.

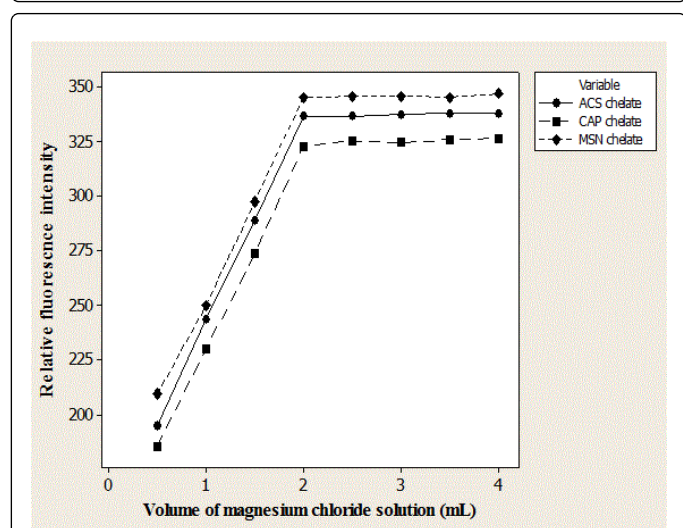


Figure 5: Effect of the volume of magnesium chloride solution on the fluorescence intensity produced from ACS, CAP and MSN.

Validation of the proposed method

Linearity and concentration ranges: Under the above described experimental conditions, linear correlations were observed by plotting drug concentrations against the intensity of fluorescence for each compound.

The corresponding concentration ranges are stated in Table 1. The good linearity was evident from the low values of the standard deviation of the slopes and the high values of the correlation coefficients.

Accuracy: The accuracy of the proposed method was assessed by analyzing each drug at various concentrations within its working range. Satisfactory recoveries % with small RSD% was obtained, indicating the high accuracy of the method.

The recovery of the drugs was calculated by performing standard additions to regular tablet excipients (lactose, microcrystalline cellulose, magnesium stearate, croscarmellose sodium and crospovidone), to regular ampoule excipients (propylene glycol, sodium metabisulphite, benzyl alcohol, mannitol) and to regular sachet excipients (aspartame, saccharine sodium, potassium bicarbonate, menthol and sucrose), where good results were obtained.

The accuracy of the proposed method was further confirmed by comparing the results of the assay of the pharmaceutical preparations with reported HPLC methods [1] for ACS and CAP and reported titrimetric method [3] for MSN by student's t-test (Table 2).

Item	ACS	CAP	MSN
λ_{em}	485 nm	485 nm	485 nm
λ_{ex}	345 nm	345 nm	345 nm
Concentration range ($\mu\text{g/mL}$)	0.04-0.44	0.04-0.44	0.04-0.44
Regression equation			
Intercept (a)	118.2	92.4	176.51
Variance of the intercept (S_a^2)	2.88	3.25	3.41
Slope (b)	1240.7	1156.2	1235.17
Variance around the slope (S_b^2)	10.44	11.78	12.34
Correlation coefficient (r)	0.9999	0.9998	0.9998
Variance (S y/x)	3.49	3.94	4.13
Accuracy (mean \pm SD %)	97.11 \pm 0.32	101.25 \pm 0.44	100.58 \pm 0.26
Precision (RSD %)	0.65	0.48	0.40
Limit of detection ($\mu\text{g/mL}$)	1.52×10^{-3}	1.48×10^{-3}	1.45×10^{-3}
Limit of quantitation ($\mu\text{g/mL}$)	4.88×10^{-3}	4.23×10^{-3}	4.75×10^{-3}

Table 1: Validation data for the determination of acetylcysteine, captopril and mesna by the proposed spectrofluorimetric method.

Precision: In order to study the precision of the proposed method, three different concentrations from the standard solutions of the investigated drugs were reacted and measured thrice. The mean relative standard deviations are presented in Table 1.

Selectivity: The selectivity of the proposed method was assessed by calculating the recovery of the drugs in presence of their respective disulfides, which were prepared by treating 5 mL of the stock solution with 5 mL peroxyacetic acid solution at 50°C for 15 min. The disulfides did not undergo the reaction thus proving the selectivity and the stability-indicating aspect of the method.

Limit of detection and limit of quantitation: The Limits of Detection (LOD) and the Limits of Quantitation (LOQ) were calculated as $3\sigma^{-1}$ and $10\sigma^{-1}$, respectively, where " σ " is the standard deviation of blank and b is the slope. The low values obtained (in the nanogram level) indicate the high sensitivity of the method.

Robustness: The robustness of the proposed method was evaluated by performing intentional slight variations of some parameters and examining their influence on the fluorescence intensity obtained. It was found that variations in the reaction time (± 2 min), in magnesium ion concentration ($\pm 5\%$), in palladium chelate concentration ($\pm 5\%$) and in pH (± 1 pH unit) had no significant effect on the measured fluorescence. RSD% of the measured fluorescence intensities after application of these variations did not exceed 2% thus indicating the robustness of the method (Table 2).

Item	Proposed method	Comparative method [1,3]
ACC tablets (200 mg acetylcysteine/tablet)		
Recovery %	101.12	100.36
	100.02	100.54
	100.96	99.19
	99.82	100.78
	100.33	100.91
SD	0.57	0.69
t*	0.19	
F*	0.69	
ACC sachets (200 mg acetylcysteine/sachet)		
Recovery%	101.2	99.55
	100.36	100.18
	101.02	100.52
	101.68	99.01
	100.86	100.76
SD	0.48	0.72
t*	2.08	
F*	0.45	
Capoten tablets (25 mg captopril/tablet)		
Recovery %	100.01	99.68
	99.96	99.84
	100.16	100.22
	99.88	99.67
	100.58	99.38
SD	0.28	0.31
t*	1.05	
F*	0.82	

Table 2: Assay results of acetylcysteine, captopril and mesna in their pharmaceutical preparations by the proposed spectrofluorimetric method.

Analysis of pharmaceutical dosage forms: The analysis of acetylcysteine, captopril and mesna in commercial dosage forms was performed using the proposed method and official methods [1,3]. The results obtained were compared statistically by the student's t-test and variance ratio F-test (Table 2). The experimental values did not exceed the theoretical ones thus, indicating the absence of any significant difference between the compared methods. Figures 6-8 shows the emission spectra of the fluorescent chelates of the three drugs in their pharmaceutical preparations.

Analysis of the drugs in plasma and urine

The proposed spectrofluorimetric method has been successfully applied to the analysis of plasma and urine samples spiked with ACS, CAP and MSN. The results are presented in Tables 3-5. Figures 9 and 10 shows the emission spectra of ACC and CAP, as representative examples, in plasma and urine.

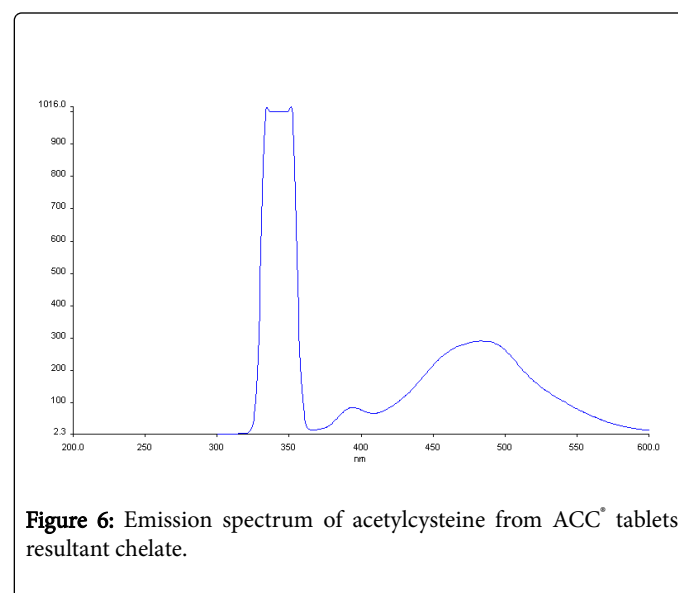


Figure 6: Emission spectrum of acetylcysteine from ACC[®] tablets resultant chelate.

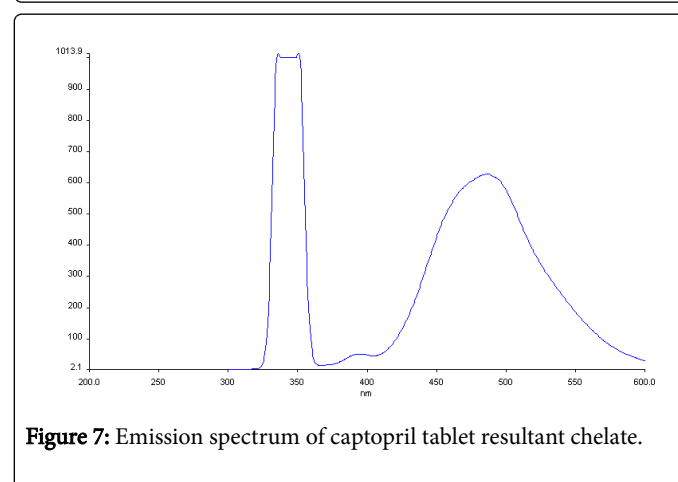


Figure 7: Emission spectrum of captopril tablet resultant chelate.

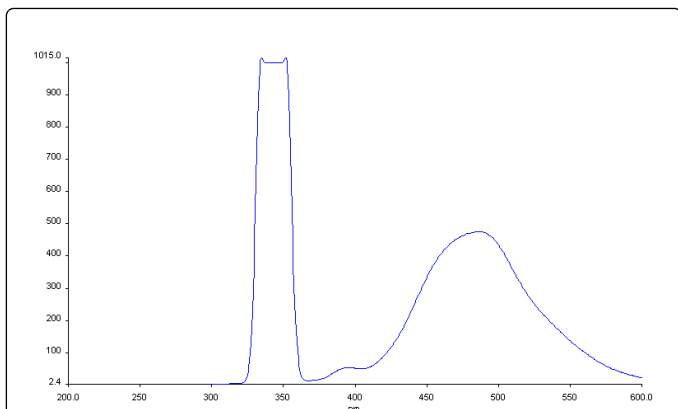


Figure 8: Emission spectrum of mesna from uromitexan[®] ampoules resultant chelate. Expired MSN ampoules were also analyzed where a decrease in a concentration (about 10%) was noticed (Table 2) which indicates the ability of the method to analyze the intact drug only and not to respond to other degradation or related products which may be produced by time on the shelf.

SD	0.54	0.26
t*	1.8	
F*	4.4	
Expired Uromitexan ampoules (400 mg mesna/ampoule)		
Recovery%	90.12 89.15 88.47 90.26 89.94	-
SD	0.76	0.96
t*	2.09	
F*	0.62	

Table 3: Recovery of acetylcysteine from plasma and urine samples by the proposed spectrofluorimetric method. *Theoretical values for t- and F- at p=0.05 are 2.31 and 6.39, respectively.

Valid Uromitexan ampoules (400 mg mesna/ampoule)		
Recovery%	101.22 100.56 100.26 101.09 99.96	99.68 99.86 100.01 100.29 99.68

Plasma			Urine		
Added (µg of ACS/mL of plasma)	Found* (µg of ACS/mL of plasma)	Recovery %	Added (µg of ACS/mL of urine)	Found* (µg of ACS/mL of urine)	Recovery %
4	3.70	92.36	45	43.03	95.62
8	7.50	93.69	90	85.37	94.86
12	11.20	93.52	130	123.64	95.11

Table 4: Recovery of captopril from plasma and urine samples by the proposed spectrofluorimetric method. *Average of triplicate samples.

Plasma			Urine		
Added (µg of CAP/mL of plasma)	Found* (µg of CAP/mL of plasma)	Recovery %	Added (µg of CAP/mL of urine)	Found* (µg of CAP/mL of urine)	Recovery %
3.5	3.28	93.68	8	7.13	89.18
7	6.52	93.13	16	14.16	88.53
10.5	10.02	95.44	24	21.60	90.02

Table 5: Recovery of mesna from plasma and urine samples by the proposed spectrofluorimetric method. *Average of triplicate samples.

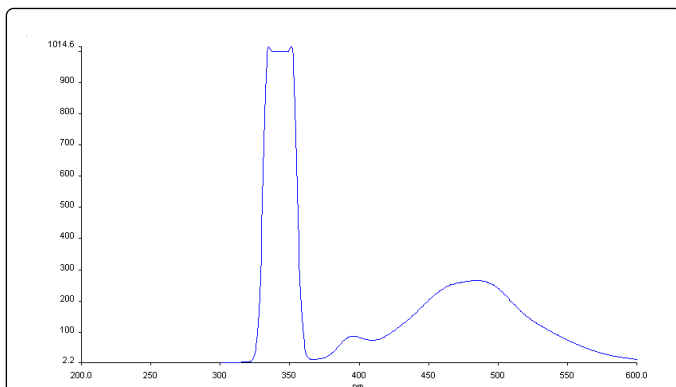


Figure 9: Emission spectrum of acetylcysteine in plasma.

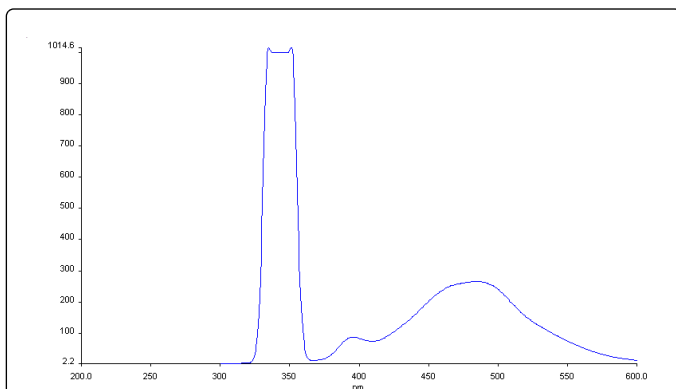


Figure 10: Emission spectrum of captopril in urine.

Conclusion

In the present work, a simple, rapid and sensitive spectrofluorimetric method was proposed for the determination of three sulfhydryl-containing drugs namely MSN, ACT and CAP, in bulk powder, in dosage forms and in biological fluids. The method is based on the demasking reaction of the palladium chelate of 8-Hydroxyquinoline-5-sulfonic acid and the subsequent formation of the corresponding fluorescent magnesium chelate that is a measure of the amount of sulfhydryl containing drug analyzed.

Although very few spectrofluorimetric procedures were found in the literature for the assay of MSN, ACT and CAP, however, there was still a need to make some improvements to the previously published methods, in order to achieve better selectivity and analytical performance. In this context, the proposed spectrofluorimetric method offers many advantages that render it superior to other reported methods. Of these we can mention its high sensitivity and its ability to analyze the drugs in nanogram levels. Another noted advantage is simplicity as no heating or tedious extraction steps were needed, in addition to rapidity and ease of automation. Finally the proposed method is stability-indicating by virtue of its capacity to selectively analyze the studied drugs in presence of their disulfides which are the potential related products or impurities.

The proposed method was successfully applied to the analysis of the three drugs in their pharmaceutical preparations, in spiked plasma and

urine samples, and therefore it can be applied in the biomedical field to study the pharmacokinetics of the investigated drugs in different formulations and combinations.

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