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Simultaneous spectrophotometric determination of clomipramine hydrochloride in pure and pharmaceutical forms with some sulfonthalein dyes

Samah A.Mohamed[1]*, Mohamed F.El-Shahat[2], Wagiha H.Mahmoud[2]
[1]National Organisation for Drug Control and Research (Nodcar) Cairo, (EGYPT)
[2]Chemistry Department, Faculty of Science, Ain-Shams University, Cairo, (EGYPT)

ABSTRACT

A simple and sensitive spectrophotometric method has been developed for the determination of clomipramine hydrochloride (CPH) in pure and tablet dosage forms by ion-pair complex formation between sulphonphthalen dyes: clomipramine hydrochloride (CPH). The method was based on the ion pair formation of clomipramine with dyes. The method was based on the formation of ion-pair complex which was extracted and measured spectrophotometrically. The ion-pair chromogen being extractable with chloroform could be measured quantitively at 420nm against reagent blank treated similarly. Beer's law is obeyed in concentration range 2-24, 2-30, 1-10 and 2-25 μg mL^{-1} with correlation coefficient equal 0.996, 0.997, 0.995 and 0.999 with Bromophenol blue (BPB), Bromothymol blue (BTB), Bromocresol purple (BCP) and Bromocresol green (BCG) respectively and Effects of foreign ions were also studied. Statistical analysis proves that the proposed method is reproducible and selective for the routine analysis of pharmaceutical formulations of Clomipramine

KEYWORDS

Clomipramine hydrochloride;
Bromophenol blue;
Bromocresol green;
Bromothymol blue.

INTRODUCTION

Clomipramine hydrochloride are a dibenzoazepine derivatives drugs belonging to class of pharmacological agents known as cyclic antidepressants (CA_s) which used to treat several psychiatric disorders, generalized anxiety disorder, depression, panic disorder, obsessive-compulsive disorder, eating disorders, attention deficit hyperactivity disorder and enuresis in children, some CA_s are available in solid oral dosage forms as either tablets or capsules[1]. Clomipramine Hydrochloride Capsules USP(25 mg, 50 mg, and 75 mg) [Mallinckrodt Inc.] Anafranil, (clomipramine hydrochloride capsules USP), is an antiobsessional drug that belongs to the class (dibenzazepine) of pharmacologic agents known as tricyclic antidepressants. Anafranil is available as capsules of 25, 50, and 75 mg[2]. The dibenzoazepine class of compounds encompasses abroad spectrum of theoretical and applied disciplines. An enormous amount of research on depressive disorders has been conducted in pharmaceutical laboratories. Dibenzoazepine derivatives are chemically active substances[3]. They react with some organic compounds and with halide and thiocyanide complexes of metals[4-6]. They also undergo oxidative reaction with the formation of colored products[7-9]. The oxidation reaction is a 4-electron process, which leads, via cation - radical intermediates, to pro-

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duce an intensity colored dimmer[10].

The therapeutical and pharmacological importance of dibenzoazepine have prompted the development of several methods for its determination including spectrochemical methods[11-14], highly performance liquid Chromatography (H.P.L.C.)[15,16], gas chromatography (G.C.)[17-19], Column Chromatography(C.C.)[20], Electrochemical methods[21-23], Capillary zone electrophoresis[24]. Nevertheless, the limit of sensitivity and the required complex equipments are common disadvantages of these methods. Ion-pair extractive spectrophotometry was used in the assay of pharmaceuticals and different alkaloids[25] due to its sensitivity and selectivity. Though, ion-pair extractive spectrophotometry has several advantages, it has some difficulties and inaccuracies due to incomplete extraction or the formation of emulsions between organic and aqueous phase. The procedure involved in the assays is totally cumbersome. Few articles[26] were published for the analysis of pharmaceutical compounds through ion-pair formation without extraction and thereby overcoming all the problems encountered in extractive spectrophotometry.

In the present work the use of bulky sulphonphthalein dyes as anionic counter ions for the formation of ion-pair complexes whose absorbance can be monitored up to extraction was investigated. The aim of present work was the development of spectrophotometric methods for determination of CPH in pharmaceutical preparations. For this purpose we studied the formation of ion-pairs between the studied drug and acid dyes and the possibility for their extraction and determination in this way. The proposed methods were demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity, and cost effectiveness.

EXPERIMENTAL

Materials and reagents

All chemicals were analytical-reagent grade (Merck, Darmstadt, Germany) and were used without previous purification. The glassware was washed with deionized water and dried. Stock solution of CPH (*see Figure 1*) at a concentration of 1000.0 μg mL^{-1} was prepared by dissolving an appropriate amount of drug (From National Organization for Drug Control and Research

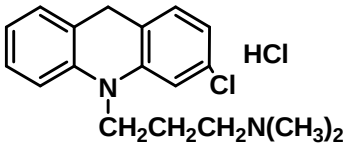

Figure 1 : Chemical structure of CPH compound

(Egypt)) in doubly distilled water. Working standards were prepared by appropriately diluting the above solutions with doubly distilled water. Bromophenol blue (BPB), bromothymol blue (BTB), bromocrysol purple (BCP) and bromocrysol green (BCG) from Aldrich used without further purification 5 x 10^{-4} M of acid dyes were prepared by dissolving the appropriate weights in little amount of acetone and continue to 100 mL of doubly distilled water. A series of buffer solution of pH 2.3-5.2, NaOAc-HCl pH (1.99, 5.2) were prepared as previously recommended[25]. All experiments were done at room temperature.

General procedures

The experimental conditions were studied separately by measuring the absorbance of the final solution resulting from the reaction mixtures containing a fixed concentration of CPH (100 μg ml^{-1}) and various amounts of the dyes (0.0 to 2.0 mL of 5 x 10^{-4} M).

A series solutions containing 0.5 mL (100 μg mL^{-1}) from base CPH, 1 mL (5 x 10^{-4} M) dyes, 1 mL buffer solutions of different pH values and 5 mL $CHCl_3$ each solution was completed to appropriate volume. The content of each flask was mixed well for 2 min., and then the extracting aqueous layer was measured against blank solution. The absorbance of the extracted drugs was measured at it is $\lambda_{max.}$ CPH solutions was measured at 406 nm with BPB and BTB, 400 nm with BCP and 407 nm with BCG.

The effect of time of ion-pair complexes formed with examined substance in pure forms was also studied at various time intervals.

To evaluate the effect of reagent concentration the drug concentration was kept constant while reagent concentration was regularly varied. The absorbance was measured at recommended wavelengths. The best reagent concentration chosen according to the highest absorbance value.

Sample preparation

The content of 20 tablets[26] was mixed thoroughly,

and the average weight of each one was determined. An accurately weighed of powder equivalent to 25 mg of CPH was transferred into 100 mL volumetric flask followed by addition of 40 mL of deionized water, the solution was ultrasonicated for 5.0 min. to dissolve and then diluted with deionized water to the mark to obtain solution of concentration 250 μg mL^{-1}. Dilute 15 mL of filtrate to 100 mL with 0.1 M HCl and measured at λ_{max}.

Suggested mechanism

The structure of the used drugs features its basic nature and suggests the possibility of utilizing an anionic dye as a chromogenic reagent. Base does not absorb in the visible region and even the dyes employed have insignificant absorbance. No ion-pair complex was formed when base was reacted with either dye but the base form did form instantaneously. Hence, the first step is the conversion of the salt to its base forms. In order to convert the hydrochloride into free base and for its quantitative extraction into the organic solvent, a series of experiments was performed. The ion-pairs formed exhibit maximum absorbance at λ_{max} as shown in Figure 2.

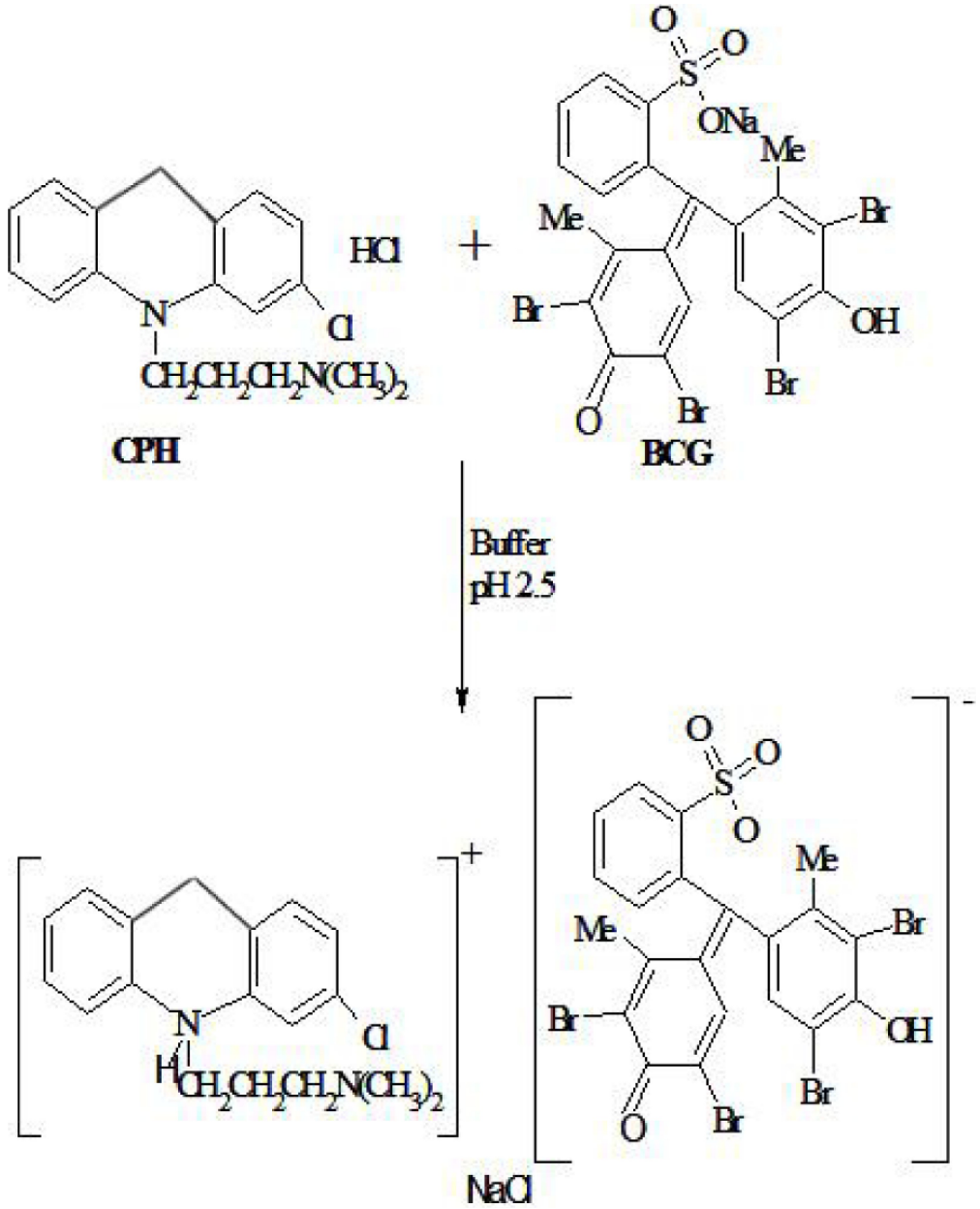

Figure 2 : Proposed mechanism of the reaction between CPH-BCG

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RESULTS AND DISCUSSION

Effect of pH on the ion-pair formation

The effect of the pH on buffer solutions was examined in the range 2.3-5.2. The results showed that the most efficient extraction of the ion-pair was obtained at pH 2.4 in case of CPH with BCP, pH 4.2 with BTB, pH = 2.5 with BCG and pH 3.9 with BPB, as shown in Figure 3. Furthermore, the amount of buffer added was examined. It is found that 1.8 mL of BPB (5 x 10^{-4} M), 1.5 mL BCG (5 x 10^{-4} M), 2 mL BTB (5 x 10^{-4}) and 2.4 mL (BCP (5 x 10^{-4} M) in case of CPH.

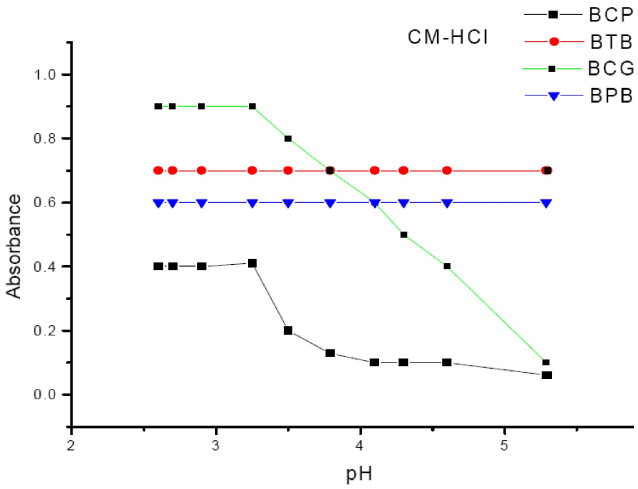

Figure 3 : Effect of the sample pH on the peak height absorbance of CPH (5.0 mg L^{-1}) with acid dyes (5 x10^{-4}M)

Effect of time

The time required for complete color development of ion–pairs formed between the drugs and acid dyes was investigated. Allowing the reaction reactants to stand and shaking for different time intervals, it was observed that 2.5 min were quite sufficient to obtain maximum color intensity before extraction of ion pair of CPH with BCG and BPB, 2 min for BCP and 3 min BTB as shown in Figure 4. the formed ion-pairs were found to be stable for more than 12.0 h.

Effect of extracting solvent

The polarity of extracting solvent effects both extraction efficiency and absorbance. The results obtained using different extracting solvents (chloroform, carbon tetrachloride, benzene, toluene, and xylene). By applying acid dyes reagent on clomipramine hydrochloride indicate that carbon tetrachloride was the best solvent for

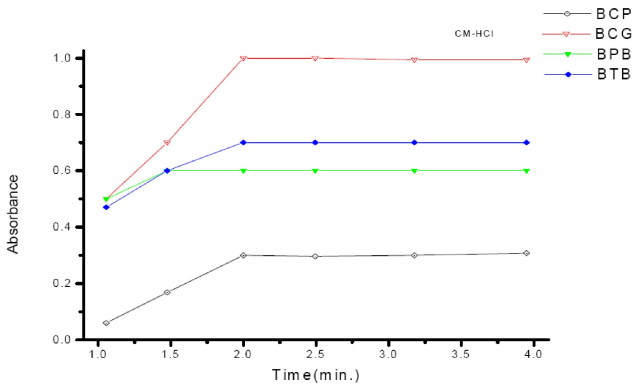

Figure 4 : Effect of shaking time on the absorbance of the CPH with acid dyes (5 x 10^{-4} M)

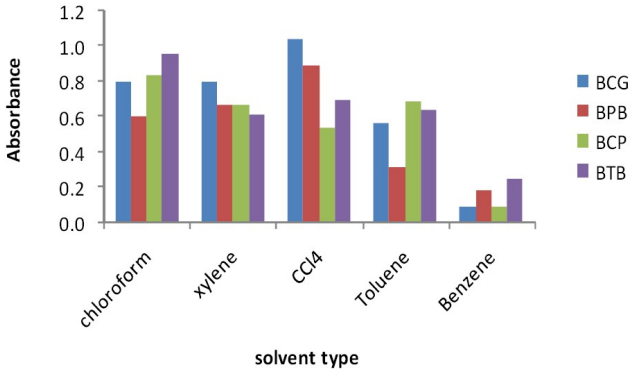

Figure 5 : Effect of extracting solvent on the absorbance of the CPH with acid dyes(5x10^{-4} M)

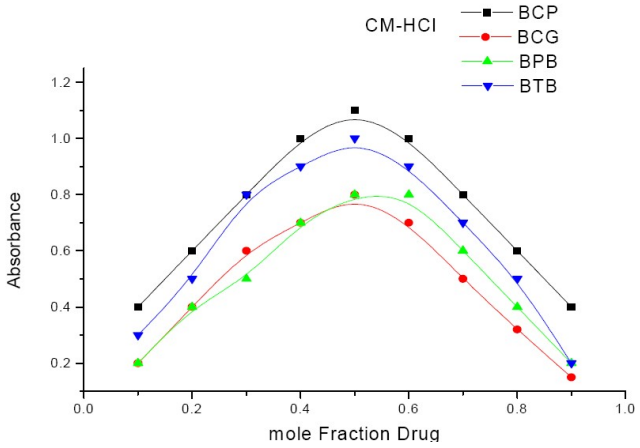

Figure 6 : Continuous variation using acid dyes (5 x 10^{-4}M) with CPH (5 x 10^{-4} M)

extraction with BPB, BCG while chloroform was the best for BTB and BCP as shown in Figure 5. The solvent was selected due to higher sensitivity and considerably lower extraction of reagent itself. Complete extraction was obtained by extraction with 5 mL of solvent for one time with an organic: aqueous phase of 1:1 is practically 100%.

Effect of reagent concentration

When various concentrations of acid dyes were

added to fixed concentration of CPH, it was observed that 2 mL of BPB, BCG, BTB (5 x 10^{-4} M) and 2.4 mL, BCP (5 x 10^{-4}M) was sufficient to produce maximum and reproducible color intensity with drugs.

Molar ratio of ion pair complexes

In order to investigate the molar ratio of the complexes formed between the CPH with acid dyes at the selected conditions, the molar ratio as shown in Figure 6 and continuous variation methods as shown in Figure 7 were carried out. The results indicated that the molar ratio of the drug to dyes was found to be (1:1) with all ion-pairs formed. The shape of the curves indicated that the complexes were labile.

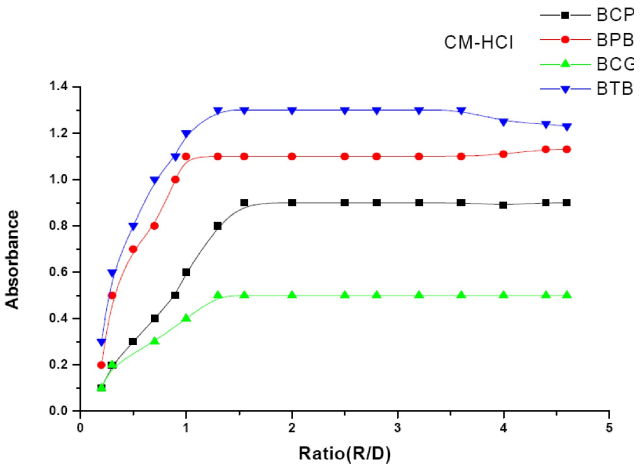

Figure7 : Molar ratio for acid dyes with CPH (5 x 10^{-4} M)

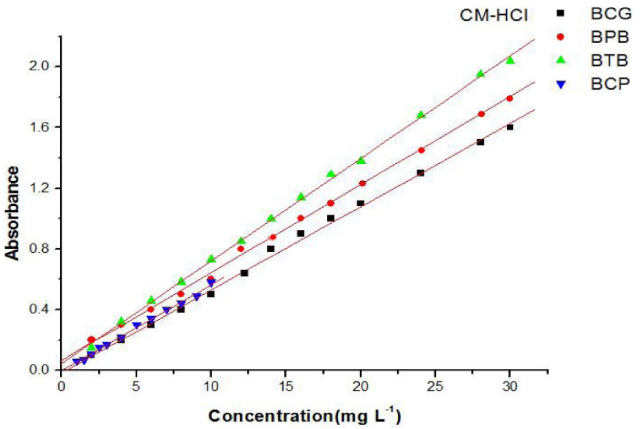

Figure 8 : Application of Beer's law for CPH using the optimum volume of acid dyes (5x10^{-4})

Analytical performance

After optimization of method the linear working ranges for CPH concentrations with BTB, BCB, BPB and BCG up to 30, 10, 20 and 30 mg L^{-1}, respectively, were obtained. The analytical curves of CPH with BCG, BPB, BCG and BTB were represented as *A= 0.052 C + 0.001, A= 0.0618 C + 0.0205, A= 0.0565C + 0.002, A= 0.0677C + 0.0438,* respectively, where *A* was the absorbance and *C* was the concentration of expressed in µg mL^{-1}, with a correlation coefficient of 0.996, 0.997, 0.995, 0.999, respectively as represented in Figure 8. The R.S.D. was <1.0% (*n* = 10).

The mean molar absorptivity, Sandllel's sensitivity, detection and quantification limits were calculated from standard deviation of the absorbance measurement obtained from Beer's law and recorded in TABLE 1.

TABLE 1 : Optical characteristic and precision data of CPH ion-pair complexes

Method	CPH			
	BCG	BPB	BCP	BTB
PH	2.4	4.3	2.4	4.3
λmax nm	407	406	400	406
Molar absorptivity (L mol–1 cm–1) x 104	1.7118	2.105	1.954	2.386
Detection-limits (µg mL-1)	0.3322	0.175	0.053	0.0443
Sandell sensitivity (µg cm–2)	0.01919	0.01618	0.0177	0.01477
SD	0.0058	0.0037	0.001	0.001
Correlation Coefficient (r2)	0.993	0.996	0.994	0.999
RSD (%)	0.553	0.93	0.302	0.206

Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of each drug was prepared and analyzed in six replicates. The analytical results obtained from these investigations were summarized in TABLE 2. The percent standard deviations and the percent range of error at 95% confidence level were calculated. The results can be considered to be very satisfactory, at least for the level of concentrations examined.

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TABLE 2 : Accuracy of the proposed methods for CPH determination by add-found test

Drugs	Reagent	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery (%)	RSD[a] (%)	RE (%)
Clomipramine - HCl	BCG	2	2.07	103.5	0.806	0.067
		4	4.24	106	0.224	0.033
		6	5.94	99	1.553	0.333
	BPB	2	1.98	99	0.806	0.068
		4	4.00	100	0.459	0.066
		6	5.89	98.23	0.934	0.207
	BTB	2	2.0	100	0.609	0.057
		4	4.0	100	0.555	0.0991
		6	5.9	99	0.206	0.0577
	BCP	2	1.92	96.2	0.139	0.0333
		4	4.0	100	0.5368	0.0667
		6	5.8	97.92	0.302	0.0577

a: relative standard deviation for six determinations

TABLE 3 : Determination of CPH in its pharmaceutical dosage (Anfranil tablets) applying standard addition technique

Drug	Reagent	Taken, mg	Recovery, %	RSD %
Anfranil (CPH)	BCG	25	102	0.1318
	BPB	25	99.04	0.192
	BTB	25	99.8	0.5853
	BCP	25	100	0.3773

TABLE 4 : Recovery of clomipramine hydrochloride in the presence of excipients and other substances

Foreign Substance	Amount (mg)	Recovery CPH[a] %
Glucose	10	101.9
Lactose	10	102.1
Mannitol	10	101.8
Fructose	10	99.8
Magnesium sulphate	10	101.16
Starch	10	99.17

a:10000 ng mL^{-1} of CPH taken

Interference

To emphasis the feasibility of the method for pharmaceutical analysis, the effects of some co-existing species found as major components in real samples were investigated. Organic substances that often accompany CPH in pharmaceutical preparations and commonly encountered pharmaceutical additives and excipients such as glucose, lactose, starch, glycine, fructose, ammonium sulfamate and some inorganic salts were examined. The tolerance levels were found relatively high (≥500 mg L^{-1}) and corresponding recovery values were quantitative (97 – 105 %) as shown in TABLE 3. The tolerance limit was deûned as the concentration of foreign substance which caused an error of ±5% in the recovery.

Application to pharmaceutical preparations

The proposed methods were applied to analysis of CPH pharmaceutical forms. The result compared statistically with official methods as shown in TABLE 4.

CONCLUSIONS

The results obtained showed that the spectrophotometric monitoring of the reaction products between clomipramine hydrochloride with acid dyes is a valuable analytical strategy for the determination of this antidepressant drug in pure and pharmaceutical preparations. When extracted with appropriate solvent it allows the development of a simple, fast and easily methodology, which could be advantageously applied in routine analyses. This assumption is reinforced by the limited interference of the substances commonly used as excipients. Moreover, it does not require any sample pre-treatment which could noticeably improve the time required for the analysis.

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