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Determination of hydrochlorothiazide in pharmaceutical formulations by diffuse reflectance spectroscopy

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Abstract: This paper describes a method for quantitative spot test analysis of hydrochlorothiazide using diffuse reflectance spectroscopy. The reflectance measurements were performed analyzing the colored compound (l = 585 nm) produced from the reaction between hydrochlorothiazide and p-dimethylaminocinnamaldehyde (PDAC) in acid medium. This reaction occurred on filter paper after heating to 80ºC for 8 minutes. Factorial designs allowed varying multiple reaction factors simultaneously in order to obtain the best reaction conditions. These factors included heating temperature, heating time, acid volume and PDAC volume. The linearity was studied in the range of $3.36x10^{-2}$ to $1.01x10^{-1}$ mol L^{-1} with a correlation coefficient of 0.998. The limit of detection was estimated to be $1.32x10^{-2}$ mol L^{-1}. Commercial samples were analyzed using the proposed method and the results were favorably compared with those of the United States Pharmacopeia method, showing that quantitative spot test analysis by diffuse reflectance could be successfully used to determine hydrochlorothiazide in medicines.

Keywords: diffuse reflectance spectroscopy; hydrochlorothiazide; spot test; p-dimethylaminocinnamaldehyde.

Introduction

Hydrochlorothiazide (6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,2-dioxide) is the prototype of the thiazide drugs. These drugs comprise an important class of diuretics. Hydrochlorothiazide is indicated for the treatment of edemas associated with the heart (congestive heart failure), liver (hepatic cirrhosis) and kidneys (nephrotic syndrome, chronic renal failure, acute glomerolonephritis). It has also been used for all degrees of hypertension, being efficient as antihypertensive agents of the other classes [1]. Hydrochlorothiazide has been used in monotherapy or in combination with other drugs such as captopril [2], losartan [3], cilazapril [4-5], benazepril [6-7], amiloride [8], fosinopril [9] and irbesartan [10].

The majority of analytical methods for the determination of hydrochlorothiazide are based on spectrophotometry [5-6, 8-13]. Spectrophotometric methods based on direct UV absorbance measurements are subject to interference from co-formulated drugs and/or tablet matrix [12], showing low selectivity since all unsaturated compounds display one or more bands in that region of the spectrum. Therefore, many methods using derivative spectrophotometry have been developed with the purpose of obtaining greater selectivity than does normal spectrophotometry [5-6, 8-11]. Nevertheless, UV derivative spectrophotometry involves complicated procedures making it limited to routine drug quality control. A UV spectrophotometric method for the determination of hydrochlorothiazide in tablets using multivariate analysis of data has been reported and considered

simple and selective. However, this kind of analysis mostly depends on the knowledge of all components present in the sample that could affect the absorbance [8]. Reversed-phase HPLC with UV detection has also been described for the determination of hydrochlorothiazide [3-4, 7]. With the aim of reducing solvent consumption and making the HPLC methods more selective, microbore columns have been used [7], but these columns are not always easily available. HPLC coupled to chemiluminescence detection has also been used for the determination of hydrochlorothiazide in tablet formulations [2, 14]. In this case the detection has been based on the reaction of hydrochlorothiazide with cerium (IV) in sulphuric acid medium, sensitized by the fluorescent dye rhodamine 6G [2, 14].

Too little attention has been given to diffuse reflectance spectroscopy as a quantitative technique as it was not possible to attain highly precise measurements from conventional spot tests [15]. However, the development of optical devices including optical fibers and reflectance spheres allowed quantification from spot tests with good precision and selectivity [16]. The diffuse reflectance spectroscopy using quantitative spot test analysis has been used for determination of ions [17-20]. With the aim of expanding the possibilities of this technique for the analysis of drugs, we developed an analytical method for the determination of hydrochlorothiazide in pharmaceutical formulations. The proposed method associates the advantages of diffuse reflectance spectroscopy (which overcomes interference problems derived from excipients present in the pharmaceutical formulations shown by UV spectrophotometric methods) and spot test analysis (which reduces solvent usage and shortens analysis time). These are important features for quality-control analysis when a great number of samples need to be analyzed in a short time.

The proposed method involved a spot test procedure based on the reaction of hydrochlorothiazide with p-dimethylaminocinnamaldehyde (PDAC) in acid medium, which occurred on filter paper at 80ºC for 8 minutes. It is assumed that in this reaction takes place the condensation of the protonated secondary amino group with carbonyl group of the reagent to produce imminium salt [21]. The factors involved in the spot test reaction included temperature, heating time and volumes of acid and reagent. These factors were evaluated and the optimum reaction conditions were chosen using factorial design.

The results obtained from the present study demonstrated the feasibly of the quantitative spot test analysis using diffuse reflectance spectroscopy, making it an interesting alternative for routine quality control of hydrochlorothiazide in tablets.

Experimental

Materials, chemicals and solutions

Whatman 42 filter paper was used as the solid support. All reagents utilized were of analytical grade. The excipients used in the interference study were of pharmaceutical grade. The solvents used were acetone (p.a. grade) and methanol (HPLC grade) from Mallinckrodt, Xalostoc, Mexico. p-Dimethylaminocinnamaldehyde (p.a. grade, Riedel-de haën, Germany) was used to prepare a 0.4% (w/v) solution in methanol and it was kept refrigerated for no more than one week. Hydrochloric acid (Mallinckrodt, Xalostoc, Mexico) at 10% (w/v) in methanol was prepared by adequate dilution of concentrated acid (37%). Hydrochlorothiazide standard was purchased from Purifarma, Brazil (purity > 99.99%). A 1.68×10^{-1} mol L^{-1} stock standard solution of hydrochlorothiazide in acetone was daily prepared. Working standard solutions were prepared by appropriate dilution of the stock solution with acetone in order to construct an analytical curve from 3.36×10^{-2} to 1.01×10^{-1} mol L^{-1}.

Pharmaceutical Formulations

The analyzed products were purchased locally and all were tested prior to the listed expiration date. Six pharmaceutical formulations containing hydrochlorothiazide and other components were analyzed. All the commercial brands of pharmaceuticals studied were package labeled to contain 50 mg hydrochlorothiazide per tablet.

Apparatus

The detection was performed by coupling a Labsphere RSA-HP-8453 reflectance sphere integrator (76 mm, 5 W halogen source) to a Hewlett Packard HP 8453A diode array spectrophotometer.

Procedure

Filter paper was cut into 2x2 cm squares. For the spot test reaction, the solutions were spotted onto Whatman 42 filter paper. Firstly, 20 mL of the analyte solution was added, then 20 mL of the acid solution and lastly, 20mL of the reagent solution. The solutions were spotted onto the center of the filter paper using a micropipette fixed in a holder according to procedure described by Tubino et al. [19]. In the sequence, the filter paper was heated to 80ºC for 8 minutes in an oven with temperature control and then the reflectance measurements were carried out at 585 nm. The blank with 20 mL of the acetone, 20 mL of the acid solution and 20 mL of the reagent solution were spotted using the same procedure.

Experimental design and statistical analysis

The entire factorial experiment was carried out in three steps. The objective of step 1 was to perform a screening of the factors that could potentially influence the reflectance response. So, fractional factorial design was used to "screen" the following factors: heating temperature, heating time and the volumes of the acid and the PDAC. The fractional factorial design was set-up in two levels, with 3 replicates. The original values of each factor were coded (1 for the high level and -1 for the low level) to remove the measurement units and to facilitate the experimental design. Table 1 shows the matrix of the experimental design generated from combinations of all factor levels.

Table 1. Matrix of the fractional factorial design.

Experiments	Factors			
	Temperature (ºC)[a]	Time (min.)[b]	Acid volume (μL)[c]	PDAC volume (μL)[c]
1	-1	1	1	-1
2	-1	-1	1	1
3	-1	-1	-1	-1
4	-1	-1	-1	1
5	1	1	-1	1
6	1	1	1	1
7	1	1	-1	-1
8	1	-1	1	-1

[a] 1 for 90ºC and -1 for 60ºC.
[b] 1 for 10 min and -1 for 5 min.
[c] 1 for 10 mL and -1 for 20 mL.

Table 2. Matrix of the full factorial design.

Experiments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Temperature (ºC)	80	90	60	60	70	80	70	80	80	70	90	60	90	90	60	70
Time (min)	5	8	7	8	5	7	7	8	10	8	5	5	10	8	10	10

Based on the results of step 1, step 2 was carried out in order to evaluate the effect of the most significant factors on the reflectance response. The full factorial design comprised four levels of temperature and heating time, ranging from 60 to 90ºC and from 5 to 10 min, respectively. Table 2 shows the matrix created from the full factorial design. All factorial designs were outlined using Statistica 5.0 software (serial number SW7127999218G51).

In step 3, the results obtained from experimental design relative to step 2 were fitted to a response surface to generate a global optimum reaction condition. The software (Statistica 5.0) fitted the factors to a complete quadratic response surface and analyzed the fitted surface to determine which values of each of the factors tested would give an optimum reflectance response.

Interference study

Since the aim of this study was to determine hydrochlorothiazide in pharmaceuticals, the effects of the most commonly used excipients were carefully examined. The excipients studied were starch, talc, magnesium stearate, lactose, ethylcelulose and silicon dioxide. For this study, solutions containing hydrochlorothiazide and each of the excipients, taken separately, in concentrations equal or ten-times greater than that of hydrochlorothiazide were shaken with acetone in a magnetic mixer for 10 minutes, diluted and analyzed under the same conditions described before, in the Procedure section.

Sample preparation

Twenty tablets of each commercial pharmaceutical brand to be studied were weighed and finely powdered. A portion of this powder, equivalent to c.a. 150 mg of hydrochlorothiazide was accurately weighed. The sample was shaken with acetone in a magnetic mixer for 10 minutes and filtered in Whatman 41 filter paper. This solution was then diluted with acetone in a calibrated 10 mL flask and an aliquot of this solution was taken for the spot test reflectance analysis.

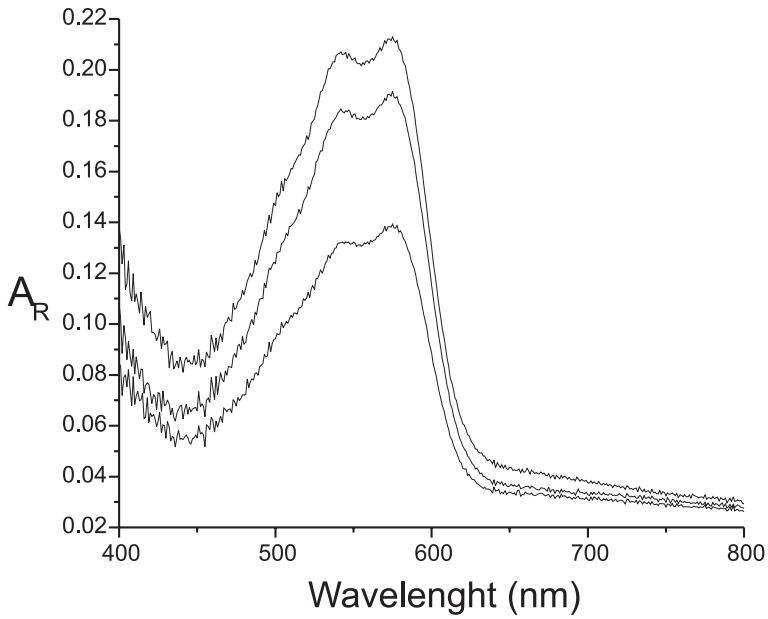

Figure 1. Reflectance spectrum of the spot test reaction on filter paper from hydrochlorothiazide and PDAC, in HCl medium. A_R values were taken after heating to 80ºC for 8 minutes. The maximum value of A_R was at 585 nm. Hydrochlorothiazide concentrations: a) 1.01×10^{-1} mol L^{-1}; b) 6.72×10^{-2} mol L^{-1}; c) 3.36×10^{-2} mol L^{-1}.

Results and discussion

PDAC has demonstrated a wide usefulness as a chromogenic reagent for spectrophotometric analysis of several compounds such as urea, thiourea and their N-alkyl/aryl derivates [22], aceclofenac [23], sodium diclofenac [21], oxyphenbutazone [24], glafenine and metoclopramide [25]. PDAC has also been used for microplate-based assay of p-aminohippuric acid [26] in plasma and urine samples, and for rapid testing of benzodiazepines [27] in pharmaceuticals. The use of a modified dimethylaminocinnamaldehyde has also been described for analysis of flavanols in wines [28]. The majority of the methods mentioned above involve a reaction in acid medium with heating to produce colored compounds (ranging from orange to red or pink). In our study a pink color appeared on the surface of the filter paper indicating the production of the compound. Figure 1 shows the reflectance spectrum with maximum value of A_R (optical density for reflectance measurement) at 585 nm.

Experimental design and statistical analysis

Step 1. Fractional factorial design: screening the factors

Presuming that the spot test reaction can be affected by multiple factors involved in the reaction; we investigated the most favorable conditions for the reaction on filter paper in order to achieve maximum color development at 585 nm. Fractional factorial designs allowed the simultaneous study of the effects that various factors might have on the spot test reaction. The factors evaluated were: heating temperature, heating time and the volumes of acid and reagent. The fractional factorial study revealed that the estimated effects obtained from temperature and time factors were greater than those from volumes of acid and PDAC. Thus, temperature and heating time were considered to be more important factors than acid and PDAC volume since these two factors have greater influence on the color response in the spot test reaction.

Step 2. Full factorial design: effects of temperature and heating time

The study of the effects of the temperature and heating time on the spot test reaction was not easily executed since the paper does not resist high temperatures (near 90ºC) and signals of carbonization began to appear and a different reflectance spectrum was displayed, such that the matrix showed in Tables 1 and 2 could not be fully carried out. The heating of the reaction on the filter paper at 90ºC could not be sustained for more than 5 minutes. So, the combinations that involved a temperature of 90ºC for 7, 8 and 10 minutes could not be performed.

Step 3. Response surface

Response surface methodology (RSM) is a powerful experimental tool for optimizing multiple and interrelated parameters. Implementing this method, experiments were conducted to find which values of the factors (referred to as independent variables) optimize the reflectance response (dependent variable). The values of the dependent variable (reflectance response) were measured for each independent variable (temperature and heating time) condition. Once this was done, a quadratic response surface can be obtained. But, the optimum value for the reflectance response could not be predicted from the estimated surface since the model did not showed F-test significance. Even so, the response surface obtained was useful to observe that the predicted optimum region was far away from the experimental region. Moreover, the shape of the surface analyzed indicated the direction in which new experiments should be performed. Therefore, due to experimental limitations (higher temperatures and longer times), the factorial design could not be extended to other areas of the surface.

Figure 2 shows the three-dimensional graph obtained from the experimental data and fitted to a response surface. Analyzing the fitted surface, it is possible to see the points referring to the conditions chosen, which showed the highest reflectance values.

As can be see in Figure 2, the highest reflectance response of the spot test was reached by heating to 80ºC for 8 minutes. It is well known that the order of addition of the solutions in the spot test reaction can affect the uniformity of the spot test color over the entire surface and the color intensity [19-20, 29]. So, we were concerned about taking care with this detail and studied all possible combinations of addition of the solutions. It was noted that uniform and intense color spots were obtained when the solution of analyte was added first followed by the acid solution and lastly, the reagent solution.

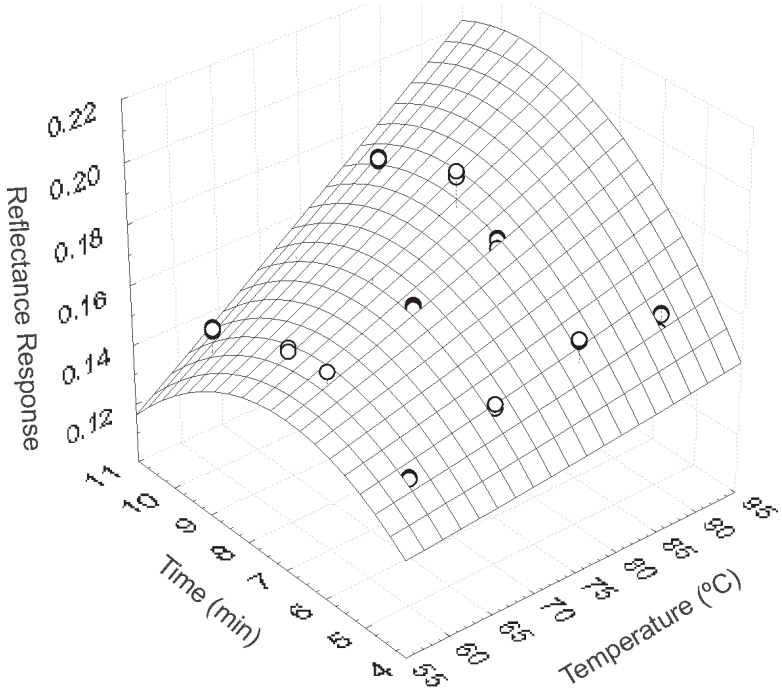

Figure 2. Surface response obtained to establish the best conditions of temperature and heating time.

Analytical characteristics

The calibration curve was constructed from standard solutions of hydrochlorothiazide with concentrations of 3.36×10^{-2} to 1.01×10^{-1} mol L^{-1}. A linear relationship (r = 0.998) was obtained by plotting A_R vs. log concentration of hydrochlorothiazide (mol L^{-1}). A_R values for the concentration range were fitted by the equation: $A_R = -0.13487 + 0.17488 \times C$, where C = log ($10^3$ [Hydrochlorothiazide] / mol L^{-1}). The factor 10^3 was used to adjust the calibration graph to log values higher than zero. The detection limit was estimated to be 1.32×10^{-2} mol L^{-1}, according to the analytical curve data and using the criteria of the mathematical model given by Miller and Miller [30].

Application of the proposed method

The proposed method was applied to some commercial pharmaceuticals containing hydrochlorothiazide. The results obtained by the reflectance method showed good agreement with those of the pharmacopeial method, which is based on UV spectrophotometry [31] (Table 3). For all formulations assayed, the results obtained by the pharmacopeial and proposed methods were compared by applying the F-test and t-test at 95% confidence level. In all cases, the calculated F and t values did not exceed the theoretical values, indicating that there is no significant difference between either method regarding precision and accuracy in determining hydrochlorothiazide in these pharmaceuticals. The average recoveries for the pharmaceutical formulations obtained by the proposed method ranged from 96.9-103.0%, whereas for the pharmacopeial method they ranged from 95.5-101.0%.

Table 3. Determination of hydrochlorothiazide in commercial pharmaceuticals.

Samples[a]	Proposed Method				Official Method [32]	
	Found[b] (mg/tablet)	Recovery (%)[b]	t-Value (2.78)[c]	F-Value (19.00)[c]	Found[b] (mg/tablet)	Recovery (%)[b]
A	49.2±2.2	98.3±4.5	1.04	1.92	49.5± 0.5	98.9±1.1
B	48.5±1.8	96.9±3.5	0.11	1.82	48.3±1.3	96.7±2.6
C	49.5±2.6	99.1±5.2	0.62	13.99	48.6± 0.7	97.1±1.4
D	51.5±2.6	103.0±5.2	0.53	1.68	50.5± 2.0	101.0±4.0
E	50.0±1.0	100.1±1.9	1.62	5.53	49.1±0.4	98.8±0.3
F	48.8±1.7	97.7±3.4	0.69	1.61	48.1±2.7	95.5±4.3

[a] All packages labeled to contain 50 mg hydrochlorothiazide/tablet. These contain many or all of the following excipients: starch, talc, magnesium stearate, lactose, ethylcelulose and silicon dioxide.
[b] Average ± Standard deviation (SD), n = 3.
[c] Theoretical values of t and F at 95% confidence level.

Conclusions

This method showed itself to be a reliable alternative to the other procedures existing for hydrochlorothiazide analysis in tablets. Factorial designs showed that temperature and heating time were the most important factors that affect the spot test reaction and the optimal temperature and heating time conditions were established from a response surface. The best conditions for the colored spot test reaction were obtained from 20mL of hydrochlorothiazide, 20 mL of hydrochloric acid solution and 20 mL of PDAC solution in this addition order, heated to 80ºC for 8 minutes. This method was successfully applied to the analysis of hydrochlorothiazide in six commercial brands of tablets. Comparing with other reported methods for hydrochlorothiazide analysis in tablets, the proposed method has advantages concerning to simplicity, low-cost and rapidity, fulfilling the requirements for the routine quality control.

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M.A. Gotardo, L. Pezza, H.R. Pezza. Determinação de hidroclorotiazida em formulações farmacêuticas por espectroscopia de reflectância difusa.

Resumo: Este trabalho descreve um método por espectroscopia de reflectância difusa utilizando spot test quantitativo para a análise de hidroclorotiazida. As medidas de reflectância foram realizadas analisando-se o composto colorido (l = 585 nm) produzido a partir da reação entre hidroclorotiazida e p-dimetilaminocinamaldeído (PDAC) em meio ácido. Esta reação ocorreu sobre papel de filtro após aquecimento a 80ºC por 8 minutos. Planejamentos fatoriais permitiram variar simultaneamente múltiplos fatores envolvidos na reação visando obter as melhores condições da reação. Tais fatores incluíram: temperatura e tempo de aquecimento, volume de ácido e volume de PDAC. A linearidade foi estudada na faixa de $3{,}36x10^{-2}$ a $1{,}01x10^{-1}$ mol L^{-1} com um coeficiente de correlação de 0,998. O limite de detecção foi estimado em $1{,}32x10^{-2}$ mol L^{-1}. Amostras comerciais foram analisadas utilizando-se o método proposto e os resultados foram favoravelmente comparados àqueles obtidos pelo método da Farmacopéia Americana, mostrando que a análise por reflectância difusa utilizando reações de spot test pode ser empregada com sucesso para determinar hidroclorotiazida em medicamentos.

Palavras-chave: espectroscopia de reflectância difusa; hidroclorotiazida; spot test; p-dimetilaminocinamaldeído.

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