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Stability-Indicating Densitometric Determination of Some Antidiabetic Drugs in Dosage Forms, Using TLC

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Abstract. The most commonly used antidiabetic sulfonylurea drugs, gliclazide, glipizide and glibenclamide, were determined using stability-indicating densitometric methods. The degradation products were prepared by acid hydrolysis of the intact drugs. Thin layer chromatography was carried out using silica gel 60 F_{254} plates and different mobile phases, followed by scanning of the developed chromatograms. Mixtures of the investigated drugs and their degradation products were prepared and analysed using the proposed methods, with recovery in the range 100.4–101.0% and RSD in the range 0.6–0.7%. Pharmaceutical dosage forms were assayed and found to give results of the same accuracy and reproducibility as official or reference methods.

Key words: antidiabetic drugs, stability-indicating assay, densitometric analysis, chromatographic techniques.

Gliclazide, glipizide and glibenclamide may be determined in pure form, in pharmaceutical preparations and in biological fluids by several analytical techniques including HPLC [1–7], TLC [8], GLC [9], radioimmunoassay [10, 11], spectrophotometry [12–16] and titrimetry [17, 18].

Aging or unsuitable storage conditions of the investigated drugs may lead to degradation, and, hence, lowering of the therapeutic effect. The published methods include only one TLC [8] stability-indicating method for the estimation of glibenclamide after elution from silica gel. Quantitative analysis of drugs after elution is not always quantitative, since complete removal from the stationary phase often is not achieved. Therefore determination of the substance while it is still on the plate using automated spectrodensitometry is more convenient and more sensitive. In this method the lane of interest passes under a beam of light of a certain wavelength which is absorbed by the compounds on the plate, the change in intensity of the beam is measured and the results are presented on a chart as a series of Gaussian peaks which can be integrated and quantitated [19].

The literature does not include any stability-indicating TLC assays for gliclazide and glipizide; the proposed methods are aimed at establishing new automated densitometric methods for the estimation of the investigated drugs in presence of their degradation products.

Experimental

Apparatus

Shimadzu CS-9000 dual-wavelength scanning densitometer and precoated TLC silica gel 60 F_{254} plates (Macherey–NaGel) were used.

Pure Samples

Gliclazide was kindly supplied by Les Laboratories Servier, France. The purity was found to be 99.78% according to Clark's method [20]. Glipizide was kindly supplied by Cid Co., Cairo. The purity was found to be 100.35% according to the BP (1993) method [21]. Glibenclamide was kindly supplied by Hoechst Orient, Cairo. The purity was found to be 100.47% according to the BP (1993) method [22]. Cyclohexylamine (Prolabo) is a degradation product of glipizide and glibenclamide; the purity was labelled to be 99%

Market Samples

Diamicron (Les Laboratories Servier) contained 80 mg of gliclazide per tablet. Minidiab (Cid Co.) contained 5 mg of

glipizide per tablet. Doanil (Hoechst Orient) contained 5 mg of glibenclamide per tablet. Euglucon (Boehringer Mannheim) contained 5 mg of glibenclamide per tablet.

Solvents and Mobile Phases

All solvents were of analytical grade (Merck). Chloroform–methanol (90: 10 *v/v*) was the mobile phase for gliclazide and glipizide. Chloroform–cyclohexane–glacial acetic acid–ethanol (9:9:1:1 *v/v*) was the mobile phase for glibenclamide.

Standard Solutions

Prepare a series of solutions containing 0.3–4 mg ml^{-1} gliclazide, 0.1–2.0 mg ml^{-1} glipizide or 0.4–2 mg ml^{-1} glibenclamide, using equal volumes of methylene chloride and methanol for gliclazide and glibenclamide or equal volumes of chloroform and methanol for glipizide.

Preparation of the Degradation Products

Acid hydrolysis: Transfer 100 mg of gliclazide, glipizide or glibenclamide to a 250 ml conical flask containing 50 ml of 5 *N* hydrochloric acid solution, reflux for 2 h and cool to room temperature. Add 40 ml of 5 *N* sodium hydroxide solution, filter the precipitate, wash with distilled water, dry and dissolve in the solvents mentioned under "standard solutions". The filtrate in the case of gliclazide is rendered alkaline with sodium hydroxide solution, extracted twice with 20 ml of methylene chloride which is collected with the corresponding solution prepared above. Concentrate the prepared solutions under reduced pressure to about 10 ml.

Thin Layer Chromatographic Separation of the Residual Intact Drug

Apply the concentrated organic extracts to silica gel 60 F_{254} plates in a band form, parallel with 20 µl of standard solution of gliclazide, glipizide or glibenclamide. Place the plate in a chromatographic tank previously saturated for 1 h with the mobile phase, develop by ascending chromatography through a distance of 16 cm, dry the plate in air and visualize under a UV lamp at 254 nm. Scratch the spots corresponding to the degradation products, extract with the same solvents mentioned in *Standard solutions*, filter into tared beakers, evaporate and weigh the residue. The degradation products obtained in the cases of glipizide and glibenclamide are mixed with the degradation product cyclohexylamine in the ratios of 3.2:1 *m/m* and 3.6:1 *m/m*, respectively.

Laboratory-Prepared Mixtures

Prepare a series of solutions containing from 0.62–3 mg ml^{-1} of gliclazide, 0.3–1.0 mg ml^{-1} of glipizide, 0.6–1.6 mg ml^{-1} of glibenclamide and 0.3–5.6 mg ml^{-1}, 0.1–2.7 mg ml^{-1} or 0.2–5.6 mg ml^{-1} of the degradation products of gliclazide, glipizide and glibenclamide, respectively, using the same solvents mentioned in *Standard solutions*. The solutions obtained represent 10–90% degradation.

Procedures

Construction of Calibration Curves. Apply 40 µl aliquots of the standard solutions to silica gel plates, 2 cm apart and 2 cm from the bottom edge of the plate. Place the plate in a chromatographic tank previously saturated for 1 h with the mobile phase. Develop by ascending chromatography through a distance of 16 cm, dry the plate, detect the spots under the UV lamp and scan at 230, 276 and 300 nm for gliclazide, glipizide and glibenclamide, respectively.

Plot the calibration curve representing the relationship between the recorded areas under the peaks and the corresponding concentrations and calculate the regression equations.

Assay of laboratory prepared mixtures. To a high performance thin layer chromatographic plate apply a 40 µl aliquot of each of the prepared mixtures, as above, record the area under the peak and calculate the concentration from the corresponding regression equation.

Assay of Pharmaceutical Preparations

Gliclazide tablets. Thoroughly powder twenty tablets, mix and accurately weigh an amount of the powder equivalent to 150 mg of gliclazide. Transfer to a 50-ml volumetric flask and shake for 30 min with 40 ml of a mixture of methylene chloride and methanol (1:1 *v/v*). Complete to volume using the same solvent, filter and prepare a series of four solutions containing 1.7–3 mg ml^{-1} of gliclazide using the filtrate. Repeat the TLC procedure as above.

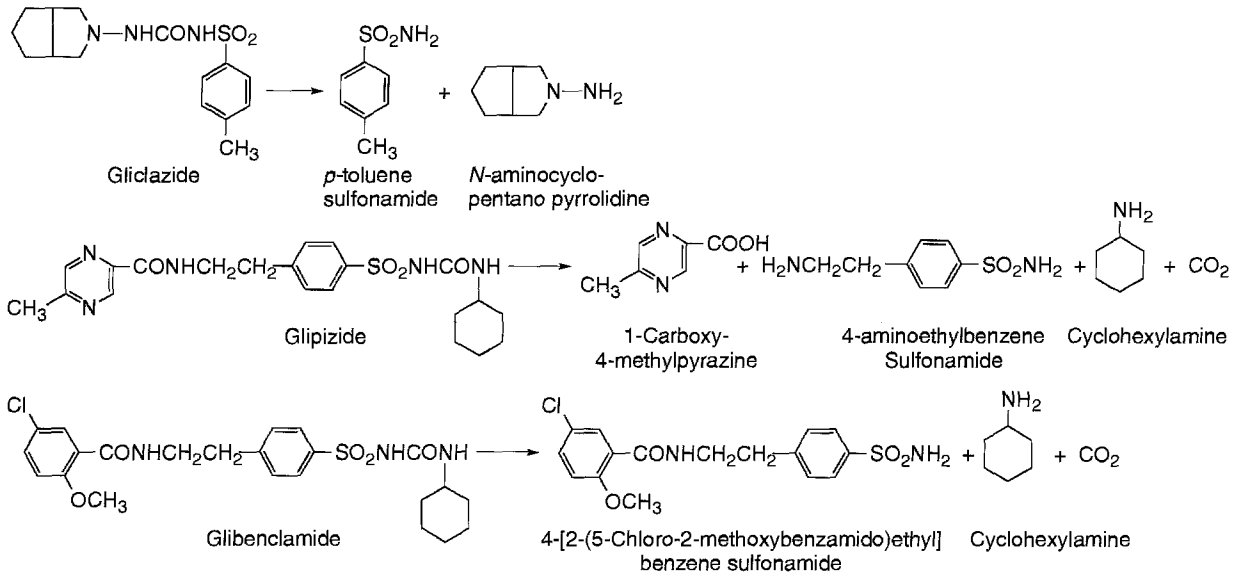
Glipizide and glibenclemide tablets. Accurately weigh an amount of the powdered tablets equivalent to 50 mg of glipizide or 100 mg of glibenclamide, shake for 30 min with 100 ml of the appropriate solvent, filter, wash with the same solvent, concentrate the filtrate and washings under reduced pressure and adjust to 50 ml in a volumetric flask. Prepare a series of four solutions containing 280–800 µg ml^{-1} glipizide or 0.5–1.55 mg ml^{-1} glibenclamide. Carry out the same TLC procedure as above.

Results and Discussion

The three investigated drugs, gliclazide, glipizide and glibenclamide, were determined in the presence of their degradation products by the densitometric technique. The degradation products were prepared via acid hydrolysis and the following equations represent the decomposition process [23–25].

Several trials for the complete separation of the intact drug from its degradation products were carried out using different mobile phases and different running distances. Complete resolution was obtained on using chloroform-methanol (90:10 *v/v*) for gliclazide and glipizide or chloroform-cyclohexane-glacial acetic acid-ethanol (9:9:1:1*v/v*) for glibenclamide. Changing the ascending distance of the mobile phase showed that 16 cm was the distance of choice.

Calibration curves were constructed applying the chosen conditions and plotting graphs for the relation between peak area and concentration of the drug over the ranges given in Table 1. Regression analysis was carried out for the slope (*b*), intercept (*a*) and



correlation coefficient (r). The results are presented in Table 1. The minimum quantifiable concentrations are 500, 150 and 450 μg ml^{-1} in case of gliclazide, glipizide and glibenclamide, respectively. The mean recoveries and S.D of within-day estimates of the minimum quantifiable concentration are 100.65±0.35, 100.1±0.58 and 99.5±0.44%, while those of between-day estimates are 101.24±0.50, 99.96±0.35 and 99.79±0.5%, respectively. It is clear that the two series of estimates are accurate and precise. To assess the validity and applicability of the proposed methods six synthetic mixtures of each drug with its corresponding degradation products in different ratios were prepared and assayed using the proposed methods (Table 2). Good recoveries were obtained, whereas those given by the official or reference methods were

Table 1. Analytical parameters for the assay of gliclazide, glipizide and glibenclamide using the proposed method

Drug	Concentration range (mg ml^{-1})	Regression equation[b]				
		Intercept (a)	Slope (b)	Correlation coefficient (r)	S. D.[a]	CV (%)
Gliclazide	0.5–3.5	0.01	24.74	1.0000	0.70	0.71
Glipizide	0.15–1.5	−0.64	62.84	1.0000	0.66	0.65
Glibenclamide	0.45–1.9	0.07	34.69	0.9985	0.60	0.59

[a] S. D of six determinations.
[b] $A = a + bC$ where A is area under peak and C is the concentration.

Table 2. Comparison between the proposed methods and the official or reference methods for the determination of the antidiabetic drugs in the presence of their degradation products

Sample no.	% of degradation product	Densitometric method[a]			Official or reference method[a]		
		Gliclazide	Glipizide	Glibenclamide	Gliclazide	Glipizide	Glibenclamide
1	10	100.78	100.54	100.63	106.50	103.00	101.71
2	20	100.00	100.63	100.69	120.59	105.85'	103.00
3	30	101.05	100.88	101.54	132.59	109.00	104.33
4	50	100.59	101.17	100.00	150.00	112.50	108.50
5	80	99.17	99.54	101.43	222.67	151.00	139.65
6	90	100.86	99.69	101.67	298.41	195.00	148.98
Mean		100.41	100.41	100.99			
S.D		0.71	0.65	0.60			

[a] % Recovery (%).

Table 3. Comparison between the results of the densitometric methods and the official or reference methods for the determination of the investigated drugs in their pharmaceutical preparations

Statistical parameter	Densitometric method				Official or reference method			
	Diamicron	Minidiab	Doanil	Euglacon	Diamicron	Minidiab	Doanil	Euglacon
Mean[a] ± S.D.	100.74±0.66	101.95±0.69	102.09±0.41	102.28±0.29	101.01±0.65	101.93±1.04	102.11±0.28	102.65±0.22
Confidence limits[b]	±0.80	±0.81	±0.45	±0.37	±0.80	±1.27	±0.34	±0.27
F-ratio[c]	1.05	2.30	2.13	1.66				
Student's t-test[d]	0.55	0.03	0.08	2.06				

[a] Mean of four determinations.
[b] Confidence limits of the mean are calculated at 95% level.
[c] Critical F-ratio value at 95% confidence level is 6.60.
[d] Critical t value at 95% confidence level is 2.45.

unacceptably high, presumably due to interference by the degradation products. The applicability of the proposed methods has been appraised further through the assay of the dosage forms. The results obtained were compared with those of the traditional UV spectrophotometric methods [20–22] using the t-test for accuracy and the F-test for assessment of precision. The calculated values did not exceed the corresponding critical values ($P = 95\%$) indicating that the difference between the results of the proposed and comparative methods was insignificant. The confidence limits of the means are also included (Table 3). Six samples can be analysed simultaneously by spotting on the same plate and results are presented on the same chart as a series of peaks. The sample throughput in the densitometric technique is higher than that of the scraping technique.

Conclusions

The results of analysis of degraded samples of gliclazide, glipizide and glibenclamide using the traditional UV spectrophotometric reference and official methods are not acceptable. The proposed methods, being stability-indicating, accurate, specific and of high precision can be recommended for the routine analysis, quality control and stability assurance of the investigated drugs in pure form and in dosage forms.

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