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Dual separation mode for simultaneous determination of antihypertensive drug combinations by high-performance liquid chromatography

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ABSTRACT

A simple, reproducible and efficient dual separation mode high performance liquid chromatographic (HPLC) method was developed for simultaneous determination of antihypertensive drug combinations including; hydrochlorothiazide (HCTZ), valsartan (VAL), amiloride (AML) and captopril (CAP). The newly developed Platinum^{TM} column, which provides a dual-mode separation with its polar and non-polar sites, was used for rapid separation of these co-administered drugs. Good resolution was obtained when Platinum^{TM} column was used compared with C_{18} column. Additionally, simple isocratic mode with mobile phase containing methanol and 0.02 mole L^{-1} phosphate buffer adjusted to pH 3.0 (45:55, v/v) was used for separation. The flow rate was 0.5 mL min^{-1} and effluent was monitored at 270 nm. All the investigated drugs were completely separated within less than 6 min. The linearity range obtained for the developed HPLC method was 0.5–100 μg mL^{-1} with detection limits of 0.13–1.2 μg mL^{-1} for all the studied drugs. The method was validated in accordance with the requirements of ICH guidelines and shown to be suitable for intended applications. The method was successfully used for determination of the studied drugs in pure form and pharmaceutical dosage forms without prior need for separation. The method is valuable for quality control laboratories for simultaneous determination of these co-administered antihypertensive drugs in binary, ternary and quaternary mixtures.

1. Introduction

Hypertension is a major risk factor for cardiovascular morbidity and mortality. Only 50% of hypertensive patients respond to monotherapy treatment [1]. Therefore, treatment of moderate or severe hypertension in most cases requires the simultaneous use of multiple antihypertensive agents. Using binary, ternary and quaternary mixtures of antihypertensive drugs with complimentary modes is more likely to achieve better blood pressure control as well as attenuate the adverse events [2]. Antihypertensive drug combinations are formulated to be administered once daily to improve the patient compliance as monotherapy, with the advantage of increased effectiveness during treatment [3].

Hydrochlorothiazide (HCTZ) (Fig. 1), is a thiazide diuretic that reduces active sodium reabsorption and peripheral vascular resistance. It is often prescribed in combination with other antihypertensive drugs such as; angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), or potassium-sparing diuretics. The combination of these drugs with HCTZ is highly efficient especially for patients not responding to monotherapy [4].

Valsartan (VAL) is a new angiotensin II receptor blocker used either alone or in combination with HCTZ [5,6]. Few methods were reported for the simultaneous determination of VAL and HCTZ in pharmaceutical dosage forms [7–10]. These methods were based on HPLC, HPTLC, capillary electrophoresis and UV-derivative spectrophotometry. Additionally, captopril (CAP) is an ACE inhibitor used for the treatment of hypertension and some types of congestive heart failure [1]. It differs from other ACE inhibitors by the presence of a sulfhydrile group. CAP is characterized by the lack of a strong chromophore and is, therefore, unable to absorb at higher wavelengths. Its binary mixture with HCTZ was determined using HPLC [11–14] and UV-derivative spectrophotometry [15]. Furthermore, amiloride (AML), a potassium-sparing diuretic, is widely used therapeutically in combination with HCTZ. AML and HCTZ mixture was analyzed in different pharmaceutical preparations by spectrophotometry [16–18], HPLC [19,20] and polarography [21]. However, the simultaneous determination of their ternary or quaternary pharmaceutical mixtures is highly desirable because it allows the generation of more efficient clinical data as well as it could be more cost-effective than separate assays. The major problem encountered during the analysis of these combinations was attributed to the strong spectral overlapping of these drugs in mixtures. This spectral interference would further complicate such analysis, especially if one of the components (HCTZ) exists as a minor relative to the other [22]. Moreover, polar drugs have

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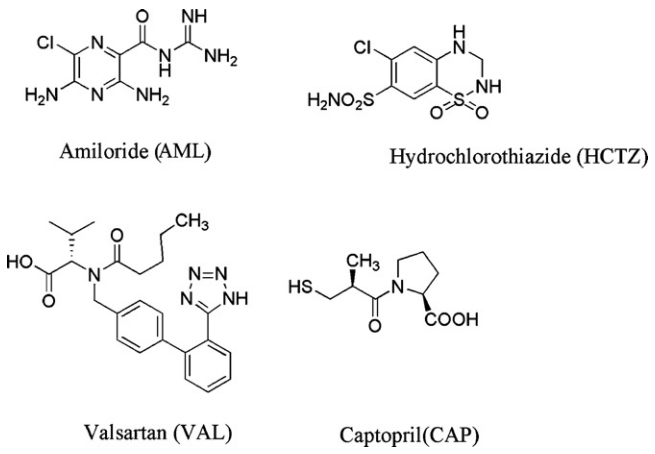

Fig. 1. Structures of the investigated antihypertensive drugs.

always been problematic in HPLC in particularly basic drugs. The major problems have been associated with the interaction between the polar drugs and C_{18} phases that make separation of complicated mixtures difficult [23].

Traditional C_{18} columns were used for separation of HCTZ in binary mixtures with VAL [7], AML [19,20] or CAP [11–14]. However, different chromatographic conditions were used in each case that may be attributed to the difference in the physico-chemical characteristics of these drugs. In addition, the obtained chromatograms had either insufficient resolution or long analysis time.

Recently, a novel approach was taken by Grace Davison Discovery Sciences® with the introduction of their Platinum™ columns. In these columns instead of thoroughly covering the silica with bonded phase (as traditional C_{18} columns), the exposure of the silica is controlled to provide a dual-mode separation with both polar and non-polar sites [24,25]. Therefore, platinum phases have enhanced polar selectivity in addition to reversed phase retention. It is proposed that the hydrophobic compounds are retained by the bonded phase (alkyl C_{18}) while the polar compounds retained by the inert silanol groups. Because of the claimed highly pure nature of the surface and the uniform covering of inert vicinal silanol groups, peak shape is generally considered to be very good [26]. Moreover, dual separation mode was successfully used for separation of some pharmaceutical mixtures [27].

In this research, we aimed to develop a simple, fast, accurate and efficient dual separation mode HPLC method using the newly developed Platinum™ column for simultaneous determination of some antihypertensive drug combinations of HCTZ, VAL, CAP, and AML in binary, ternary and quaternary mixtures. The separation mechanism was based on dual separation mode on both polar and non-polar sites of the stationary phase. The performance of platinum™ C_{18} column was compared with those of Cosmosil C_{18} column results. The method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines [28]. Then the method was applied successfully for the determination of pharmaceutical dosage forms without prior need for separation.

2. Experimental

2.1. Materials and reagents

Reference standards of HCTZ (% purity 100.3 ± 0.45) and AML (% purity 97.2 ± 0.92) were obtained from (Sigma–Aldrich, St. Louis, USA). VAL (% purity 98.1 ± 0.75) from (Novartis Pharma AG, Basle, Switzerland), CAP (% purity 100.3 ± 0.45) from (GlaxoSmithKline Co., Cairo, Egypt).

Pharmaceutical preparations containing studied drugs were obtained from the local market. Capozide®tablets (15 mg HCTZ, 50 mg CAP) were obtained from (GlaxoSmithKline Co., Cairo, Egypt), Co-Tareg®tablets (12.5 mg HCTZ, 80 mg VAL) from (Novartis Pharma Co., Cairo, Egypt), Moduretic®tablets (50 mg HCTZ, 5 mg AML) from (Cairo Pharmaceuticals Co., Cairo, Egypt) and Hydretic®tablets (12.5 mg HCTZ) from (ChemiPharm Co., Cairo, Egypt). Double distilled water was obtained using Simpli Lab-UV (Millipore, Bedford, MA, USA) water device. All other chemicals and solvents used in work were of HPLC analytical grade.

2.2. Apparatus and chromatographic conditions

A Younglin Autochro-3000 HPLC system (Younglin, Korea) with UV detector, a Rheodyne injection valve with a 20-μL loop was used. Compounds were separated isocratically on a platinum™ C_{18} column (100 mm × 4.6 mm, 3 μm i.d.) (Grace Davison Discovery Sciences®, Lokeren, Belgium) that was maintained at ambient temperature (25 °C). The performance of a platinum™ C_{18} column was tested and compared with Cosmosil 5C18-MS II (150 mm × 4.6 mm, 5 μm i.d.) (Nacalai, Japan). The mobile phase was a mixture of 0.02 mole L^{-1} phosphate buffer (pH 3.0) and methanol (55:45, v/v). The flow rate was 0.5 mL min^{-1} and detection was carried out at 270 nm. The mobile phase was filtered and degassed by sonication before use. Peak identity was confirmed by comparison of spectra and retention times with those of standards. In addition, ultrasonic cleaner (Cole-Parmer, Chicago, USA), pH meter, model 3305 (Jenway, London, UK), Sartorius handy balance H51 (Hanover, Germany) and oil-less vacuum pump (Rocker, Tiwan).

2.3. Preparation of standard solutions

A stock solution of HCTZ, AML, VAL and CAP reference standards (1.0 mg mL^{-1}) was prepared in methanol. The working standard solutions were prepared by further dilution of the stock solution with the mobile phase to obtain concentration ranging from 0.05 to 500 μg mL^{-1}. The stock and working standard solutions were kept ±4 °C in light protected flasks.

2.4. Method validation

The validation was performed according to International Conference on Harmonization (ICH) guidelines [28].

2.4.1. Linearity

The linearity of the method was checked by analyzing six solutions in the range 0.5–50 μg mL^{-1} for AML and VAL (0.5, 2, 5, 10, 25, and 50 μg mL^{-1}) and in the range 1–100 μg mL^{-1} for HCTZ and CAP (1, 2, 5, 10, 25, 50 and 100 μg mL^{-1}). Each solution was prepared in triplicate. Calibration curves were constructed as peak area versus the concentrations and the linear relationship was determined.

2.4.2. Limits of detection and quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can be readily detected but not necessarily quantified. It is usually regarded as the amount for which the signal-to-noise ratio (S/N) is 3:1. The limit of quantitation (LOQ) is defined as the lowest concentration of an analyte that can be quantified with acceptable precision and accuracy. It is usually regarded as the amount for which the S/N is 10:1. Blank and samples spiked with known concentrations of each analyte were prepared and analyzed by the proposed HPLC method. LOD and LOQ were then established experimentally by evaluating the minimum levels at which the analyte could be readily detected or accurately quantified, respectively.

2.4.3. Accuracy and precision

Intra-day precision was determined by replicate analysis ($n = 5$) of standard solutions at low, medium, and high concentration levels (5, 20 and 50 μg mL^{-1}). The inter-day precision was conducted by repeating the analysis over a period of three consecutive working days. The overall precision of the method was expressed as relative standard deviations (RSD). Method accuracy was determined by addition of known amounts of standard HCTZ, VAL, AML and CAP to a sample solution of known concentration and comparing measured and calculated values. The accuracy was expressed as percent to the true value.

2.4.4. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. For the determination of a method's robustness, a number of method parameters, for example, buffer pH, mobile phase composition, flow rate or detection wavelength, were varied within a realistic range, and the quantitative influence of the variables is determined.

2.4.5. Recovery

Recovery of HCTZ, VAL, AML and CAP from its formulations (tablets) was assessed ($n = 5$) by standard addition method. Known amounts of standard drugs were added to pharmaceutical formulations then samples were extracted (as described in Section 2.5). The obtained peak areas were compared with those obtained from direct injection of equivalent quantities of pure standards.

2.5. Analysis of dosage form

An accurately weighed amount of powder obtained from 20 tablets equivalent to 25 mg of the drug was transferred into 100 mL volumetric flask. About 50 mL methanol was added and the flask was shaked for10 min, and then was completed to 100 mL with methanol, mixed and then the solution was filtered and the first portion of filtrate was rejected. The prepared solution was diluted quantitatively to obtain the required concentration for assay. An aliquot of 20-μL was injected to HPLC system.

3. Results and discussion

3.1. Optimization chromatographic conditions

The most important aspect of method development in liquid chromatography is the achievement of sufficient resolution within a reasonable analysis time. This goal can be achieved by adjusting different chromatographic factors to give the desired response. The main analytical parameters to be optimized are the stationary phase, and mobile phase composition (organic modifier, buffer, and pH).

3.1.1. Stationary phase

Initially, traditional C_{18} column was used for separation of tested mixture HCTZ, VAL, AML and CAP but it was difficult to separate all the tested antihypertensive drugs simultaneously due to the difference in the physical–chemical characteristics of these drugs. However, binary mixtures of HCTZ with another antihypertensive drug could be separated but at different chromatographic conditions. Besides, separation took longer time or had insufficient resolution. Therefore, a column with enhanced polar selectivity was needed such as platinum^{TM} C_{18} column. The choice of the separation method depends on factors such as the nature of the drug, the complexity of the sample, and the intended use.

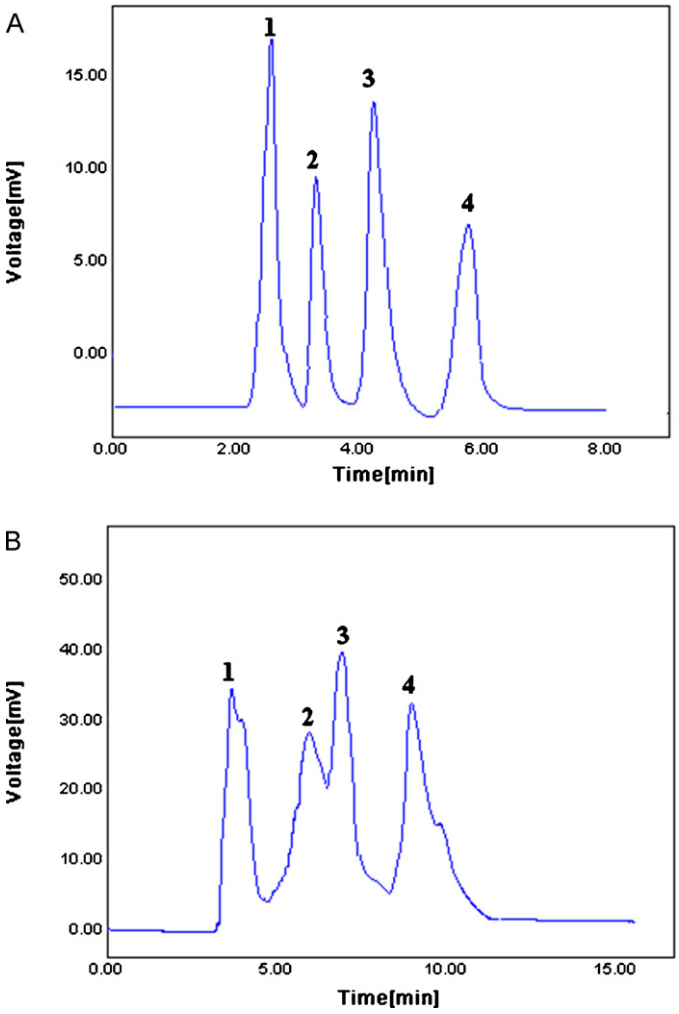

Fig. 2. Chromatograms for separation of quaternary mixture of 5 μg mL^{-1} AML (1), 10 μg mL^{-1} HCTZ (2), 40 μg mL^{-1} CAP (3) and 5 μg mL^{-1} VAL (4) using platinum^{TM} C_{18} column (A) and Cosmosil 5C18-MS II (B). Chromatographic conditions were; a mixture of 0.02 mole L^{-1} phosphate buffer (pH 3.0) and methanol (55:45, v/v), flow rate was 0.5 mL min^{-1}, injection volume; 20 μL, and UV detection at 270 nm.

It is considered important to study and understand the performance of the platinum column, which was considered as a novel stationary phase that has hydrophobic and polar selectivity providing a dual mode separation medium with both polar and non-polar sites [24,25]. Its performance was compared with traditional C_{18} columns for separation of the tested mixtures.

The mobile phase composition was modified to adapt the selected mixtures. The final optimized HPLC procedure for separation of the tested compounds was; a mixture of 0.02 mole L^{-1} phosphate buffer (pH 3.0) and methanol (55:45, v/v), flow rate was 0.5 mL min^{-1}., injection volume; 20 μL, and UV detection at 270 nm. Fig. 2 represents the chromatograms for separation of quaternary mixture of HCTZ, VAL, AML and CAP on platinum^{TM} C_{18} column (A) and Cosmosil 5C18-MS II (B). It is clear that the resolution and peak symmetry of tested mixture using platinum^{TM} C_{18} column is far better than Cosmosil 5C18-MS II and the total run time for the platinum column (6 min) is shorter than that of the C_{18} column (10 min).

The following operating parameters for the platinum column were compared with those of the C_{18} column for the tested mixtures (Table 1); retention factors (k'), number of theoretical plates (N), peak resolution (Rs), and the tailing factor ($T_{0.05}$). A good chromatographic separation requires retention factors (k'), to be neither

Table 1
Performances of platinum column and C_{18} column for separation of tested antihypertensive mixtures.

Compound	Platinum columns				C_{18} column			
	k'	$N \times 10^2$	Rs	$T_{0.05}$	k'	$N \times 10^2$	Rs	$T_{0.05}$
AML	1.27	11.2	–	1.05	1.23	4.71	–	1.54
HCTZ	2.18	16	3.1	1.15	2.65	8.07	2.8	1.8
CAP	2.91	18.8	2.3	1.08	3.17	12.60	1.05	1.3
VAL	4.27	26.6	3.75	1.10	4.4	15.64	1.89	2.5

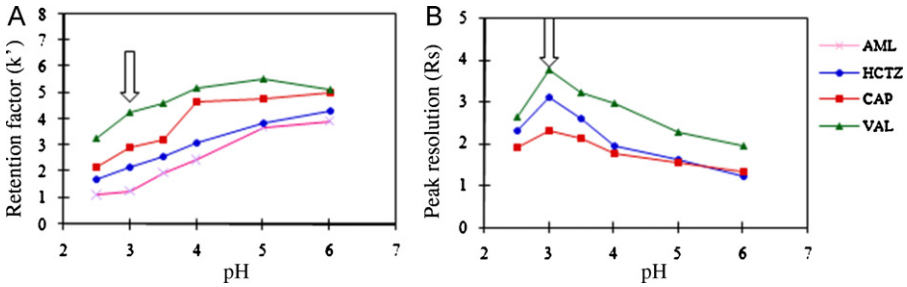

Fig. 3. Effect of buffer pH on the retention factor (k') (A) and peak resolution (Rs) (B) of tested antihypertensive mixtures using platinum column. Conditions as given in Fig. 2 except the buffer pH were varied.

too low (tendency of molecules to be in a mobile phase rather than in the stationary phase), nor too high (long analysis time, poor detection sensitivity). In addition, number of theoretical plates (N) is a measure of the efficiency of the column and tailing factor ($T_{0.05}$) is a measure of the peak symmetry. From the results presented in Table 1, it was observed that the efficiency of the platinum column with respect to the number of theoretical plates is higher than C_{18} column for all the tested antihypertensive drugs. On the other hand, the peak symmetry for the tested compounds when using platinum column is better than C_{18} column. These results make platinum column superior to C_{18} column for analysis of the tested antihypertensive mixtures that coincide with previous reports concerning separation of polar samples using platinum columns [24–26].

3.1.2. Mobile phase composition

A number of variables relating to the mobile phase composition such as buffer concentration, pH, and organic modifier were studied. Phosphate buffers are commonly used in the determination of the tested antihypertensive drugs. The effect of buffer concentration from 0.010 to 0.06 mole L^{-1} was studied. It was observed that increasing the buffer concentration provided little effect on both the retention times and the peak resolution of the tested drugs. Phosphate buffer with the concentration of 20 mmole L^{-1} was chosen for further experiments to avoid problems associated with precipitation in the presence of significant amounts of organic modifier and minimize the abrasive effect on pump seals [29].

Because the pH could alter the retention time, resolution or peak symmetry, the effect of pH of phosphate buffer was studied in pH range from 2.5 to 6 pH (Fig. 3). The retention values increased with the buffer pH increasing, but peak resolution and symmetry decreased. This behavior can be ascribed to the effects of pH on the ionization of surface silanols and the tested drugs [30]. The ionization of the silanols is gradually increased at pH more than 3 and the tested antihypertensive drugs are basic compounds with different p*K*a values are mainly in protonated state at that pH. Good retention values with acceptable peak shapes are obtained at buffer pH of 3.0, hence it was selected as the optimal pH.

The retention time and peak resolution of the studied compounds were extensively affected by the type of organic modifier used. The effect of using acetonitrile, methanol or both of them as organic modifiers was investigated. Methanol gave the best separation and peak symmetry therefore; methanol was selected as the ideal organic modifier for the investigated antihypertensive drugs. The effect of different proportions of methanol on the chromatographic behavior of the eluted peaks was studied (Fig. 4). It was observed that with reduced amounts of methanol in the mobile phase, the retention values of the studied were extended and peak resolution was decreased for all the investigated drugs. Since a short retention time is desirable provided that good resolution and peak symmetry was obtained, the proportion of 45% (v/v) of methanol was selected. It provides both short retention time and sufficient resolution for the eluted peaks.

3.1.3. The effect of the flow rate on the column efficiency

The influence of the mobile phase flow rate on the performance of the platinum columns for the separation of tested mixtures was investigated. The flow rate was varied in the range 0.3–1.5 mL min^{-1}. The Van Deemter plot (plate height (H) versus

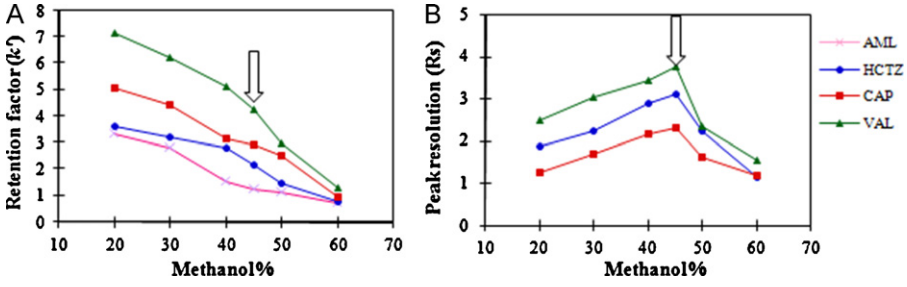

Fig. 4. Effect of methanol content on the retention factor (k') (A) and peak resolution (Rs) (B) of tested antihypertensive mixtures using platinum column. Conditions as given in Fig. 2 except the methanol content were varied.

Table 2
Retention times, calibration curves, detection and quantitation limits HCTZ, VAL, AML and CAP.

Compound	t_R (min)	Calibration curve[a] ($n=3$)				Detection limit ($\mu g\,mL^{-1}$)	Quantitation limit ($\mu g\,mL^{-1}$)
		Range ($\mu g\,mL^{-1}$)	Slope[b] (±SD)	Intercept[b] (±SD)	r		
AML	2.5	0.5–50	0.5 (±0.12)	1.1 (±0.25)	0.9995	0.13	0.43
HCTZ	3.5	1.5–100	0.4 (±0.15)	0.93 (±0.20)	0.9991	0.45	1.5
CAP	4.3	5–100	0.2 (±0.07)	1.1 (±0.25)	0.9987	1.2	4.0
VAL	5.8	0.5–50	0.5 (±0.11)	0.67 (±0.17)	0.9990	0.21	0.7

[a] Peak area of analyte versus concentration ($\mu g\,mL^{-1}$).
[b] Data presented as mean ± SD of three experiments.

Table 3
Accuracy and precision of the developed HPLC method.

Sample	Concentration ($\mu g\,mL^{-1}$)	Intra-day assay ($n=5$)		Inter-day assay ($n=3$)	
		Accuracy (%)	Precision (RSD%)	Accuracy (%)	Precision (RSD%)
HCTZ	5	100.2	0.80	100.5	1.20
	20	99.8	0.52	99.4	0.62
	50	99.1	0.40	98.7	0.75
CAP	5	98.9	0.72	99.9	1.40
	20	99.3	0.49	99.2	0.91
	50	99.6	0.82	100.3	0.70
AML	5	98.4	0.65	99.1	1.10
	20	98.9	0.74	98.5	0.89
	50	99.5	0.43	99.9	0.75
VAL	5	100.3	0.89	99.8	1.05
	20	99.1	0.68	98.8	0.92
	50	99.8	0.37	99.5	0.57

flow rate) of the tested mixtures is shown in Fig. 5. This plot shows the effect of the flow rate on the columns efficiency for separation of tested mixtures. As can be seen, the efficiency of the platinum column is not significantly affected by increasing the flow rate, but backpressure increases significantly. Therefore, flow rate of 0.5 mL min^{-1} was chosen.

3.1.4. The effect of detection wavelength

The chemical and spectroscopic behaviors of HCTZ are common knowledge. The UV absorbance spectrum of its methanol solution exhibits three absorption bands with maxima at 317, 271, and 226 nm ($A^{1\%}_{1cm}$: 130, 654, 1280) [31]. For AML, CAP and VAL, 286 nm, 210 nm, and 265 nm wavelengths were used for their detection, respectively. Therefore, effect of detection wavelengths of 226, 254, 270 and 286 nm were investigated for the tested mixture. The best detection wavelength for optimum sensitivity for most of the tested mixture was at 270 nm. However, CAP exhibits lower sensitivity of at this wavelength.

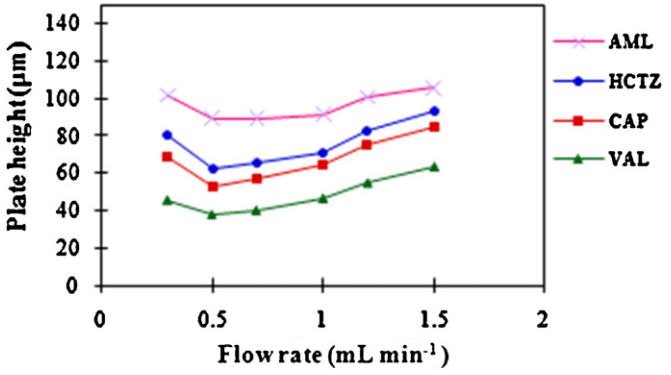

Fig. 5. Van Deemter plot of the height equivalent to a theoretical plate versus flow rate for separation of tested antihypertensive mixture using platinum column. Conditions as given in Fig. 2 except the flow rate were varied.

3.2. Validation of the developed HPLC method

3.2.1. Linearity, detection and quantitation limit

Under the optimum chromatographic conditions, the relationship between peak areas was linear in the range 0.5–50 $\mu g\,mL^{-1}$ for AML and VAL, 1.5–100 $\mu g\,mL^{-1}$ for HCTZ and 5–100 $\mu g\,mL^{-1}$ for CAP. The retention times (t_R), intercepts (a), slopes (b), correlation coefficients, limits of detections and limits of quantitations for HCTZ, VAL, AML and CAP are summarized in Table 2. All the studied analytes were fully separated within less than 6 min. Short analysis time is necessary for these frequently administered mixtures especially for quality control laboratories. The slopes of the calibration curves reflect the sensitivity of the developed HPLC method. The detection limits (S/N = 3) obtained with the developed HPLC method were 0.45, 0.21, 0.13, and 1.2 $\mu g\,mL^{-1}$ HCTZ, VAL, AML and CAP, respectively. The proposed method was found to be more sensitive than spectrophotometric methods [15–18], HPLC–UV methods [11–14], but less sensitive than LC–MS method [8]. However, the high cost and the need for technical experience make LC–MS less convenient for routine assay of the studied analytes in quality control laboratories. In addition, some of these methods require long retention times to complete the separation of all analytes in binary mixtures [11–14].

3.2.2. Precision and accuracy

Intra-day precision of the proposed method was tested by replicate analysis of five separate solutions of the working standard of HCTZ, VAL, AML and CAP at three different concentration levels; low (5 $\mu g\,mL^{-1}$), middle (20 $\mu g\,mL^{-1}$) and high (50 $\mu g\,mL^{-1}$) (Table 3). This study was repeated for three days to determine the inter-day precision. RSD % values for HCTZ, VAL, AML and CAP in all cases were ranged from 0.37 to 1.20 indicating good repeatability and precision. Accuracy was determined by comparing measured concentrations of HCTZ, VAL, AML and CAP with the actual values and expressed as percentage. The accuracy of the developed HPLC method for HCTZ, VAL, AML and CAP was ranged from 98.5 to 100.5% indicating good accuracy. The obtained accuracy and precision was satisfactory for quality control measurements

Table 4
Robustness of the developed HPLC method.

Sample	% Recovery ± SD			
	HCTZ	CAP	AML	VAL
No variations	99.7 ± 0.40	99.1 ± 0.84	98.5 ± 0.77	98.4 ± 0.64
Buffer pH				
pH 2.8	100.2 ± 0.73	98.1 ± 0.91	99.0 ± 0.65	99.6 ± 0.46
pH 3.2	98.5 ± 0.86	99.3 ± 0.62	100.7 ± 0.79	99.0 ± 0.91
Buffer conc.				
0.015 mole L^{-1}	98.7 ± 0.60	99.3 ± 0.69	99.9 ± 0.87	100.4 ± 0.55
0.025 mole L^{-1}	99.5 ± 0.75	99.8 ± 0.76	99.5 ± 0.71	99.6 ± 0.59
Methanol				
42%	99.4 ± 0.88	98.8 ± 0.95	99.8 ± 0.60	100.5 ± 0.57
48%	98.9 ± 0.75	98.3 ± 0.88	93.9 ± 0.49	99.6 ± 0.80
Flow rate				
0.7 mL min^{-1}	98.4 ± 0.81	99.1 ± 0.90	99.5 ± 0.67	99.0 ± 0.77
0.4 mL min^{-1}	99.9 ± 0.73	98.7 ± 0.83	95.9 ± 0.69	98.6 ± 0.82
Detection λ_{max}				
265 nm	98.2 ± 0.79	98.9 ± 0.56	100.1 ± 0.89	94.5 ± 0.69
275 nm	93.5 ± 0.92	99.8 ± 0.70	99.6 ± 0.98	92.7 ± 0.81

Table 5
Determination of pharmaceutical formulations by the developed HPLC method and official methods.

Product	Ingredient (content, mg)	% Recovery[a] ± SD		*F*-test[b]	*t*-test[b]
		This method	Official method[c]		
Capozide®tablets	HCTZ, 15	100.1 ± 0.92	99.2 ± 0.76	1.30	1.87
	CAP, 50	98.3 ± 1.2	99.4 ± 0.88	3.60	2.10
Co-Tareg®tablets	HCTZ, 12.5	100.8 ± 0.45	100.1 ± 0.67	2.75	1.92
	VAL, 80	99.5 ± 1.1	99.1 ± 0.73	3.31	1.95
Moduretic®tablets	HCTZ, 50	97.9 ± 0.59	98.3 ± 0.76	2.61	1.53
	AML, 5	98.9 ± 1.3	99.2 ± 0.85	3.56	2.05
Hydretic®tablets	HCTZ, 12.5	99.2 ± 0.77	99.8 ± 0.55	2.32	1.54

[a] Average of five determination ±SD
[b] Theoritical values at 95% confidence limit; $t = 2.306$, $F = 6.388$.
[c] USP 2007 [32].

for the investigated drugs in binary, tertiary and quaternary mixtures.

3.2.3. Robustness

The robustness of an analytical procedure refers to its capability to remain unaffected by small and deliberate variations in method parameters without changes in quantitation. For the determination of the method's robustness, four factors (parameters) were selected from the analytical procedure to be examined in the robustness testing: pH, mobile phase composition (organic modifier, buffer content), flow rate and detection wavelength. Results are shown in Table 4. The mobile phase composition normally affected chromatographic behavior of the investigated substances. Considering the physical–chemical characteristics of HCTZ, VAL, AML and CAP (apparent p*Ka*'s), the influence of pH of the mobile phase should be expected. However, experimental data had shown that at the ratio of methanol content from 42% to 48%, the influence of the pH of the mobile phase could be neglected. Also, detection wavelength and flow rate had a minor influence. It was found that none of these variables had a significant effect on the determination of investigated drugs. This provides an indication of the reliability of the proposed method during normal usage so the developed HPLC method considered robust.

3.3. Application of the developed HPLC method for pharmaceutical preparations

Some commercial dosage forms of the studied drugs were successfully analyzed by the developed HPLC method and results were compared with those obtained by official methods [32] as shown in Table 5. It is clear from the table that there is no significant difference between results obtained by the developed HPLC method or official methods, as indicated by *t*- and *F*-tests. Additionally, recovery experiments were carried out for the studied drugs in their respective pharmaceutical formulations by standard addition method. The results in Table 6 indicate that the extraction method is convenient for all investigated drugs with good recoveries and there is no interference from either the co-administered drug or fre-

Table 6
Percent recovery of standard drugs added to their commercial dosage forms.

Drug	Dosage form	Declared amount (mg)	Amount added (mg)	% Recovery[a] ± SD
HCTZ	Hydretic®tablets	12.5	12.5	100.3 ± 0.69
			25	99.4 ± 0.75
VAL	Co-Tareg®tablets	80	40	99.3 ± 0.54
			80	98.7 ± 0.67
CAP	Capozide®tablets	50	25	100.7 ± 1.25
			50	99.2 ± 0.95
AML	Moduretic®tablets	5	10	98.3 ± 0.88
			20	99.0 ± 0.63

[a] Average of five determinations.

quently encountered excipients. The proposed method is sensitive, accurate and precise. It is suitable for the simultaneous determination of the studied drugs in their dosage forms and application in quality control laboratories.

4. Conclusion

Herein, a simple, rapid, accurate HPLC method based on using dual separation mode of the newly introduced Platinum^{TM} columns was developed for simultaneous determination of some antihypertensive drug combinations of HCTZ, VAL, AML and CAP in antihypertensive pharmaceutical preparations. Platinum phases have enhanced polar selectivity in addition to reversed phase retention that provides high resolution for the investigated drugs in short analysis time compared with the frequently used C_{18} columns. Besides, the use of simple isocratic elution procedures for separation of co-administered antihypertensive drugs simultaneously offers a simple and convenient HPLC method for pharmaceutical industry. The method has been validated and the obtained results indicate good linearity and reproducibility over broad range of concentrations. The method is suitable for routine analysis of these pharmaceutical mixtures simultaneously in quality control laboratories.

References

[1] Goodman and Gilman's The Pharmacological Basis of Therapeutics, tenth ed., McGraw-Hill, New York, USA, 2001, p. 663.
[2] M. Destro, F. Cagnoni, A. D'Ospina, A. Ricci, E. Demichele, E. Peros, A. Zaninelli, P. Preti, Vasc. Health Risk Manag. 6 (2010) 253.
[3] S.G. Chrysant, Expert Rev Cardiovasc. Ther. 1 (2003) 335.
[4] K. Wellington, D.M. Faulds, Drugs 62 (2002) 1983.
[5] A.J. Wagstaff, Drugs 66 (2006) 1881.
[6] A. Markham, K.L. Goa, Drugs 54 (1997) 299.
[7] D.F. Tian, X.L. Tian, T. Tian, Z.Y. Wang, F.K. Mo, Indian J. Pharm. Sci. 70 (2008) 372.
[8] N.J. Shah, B.N. Suhagia, R.R. Shah, N.M. Patel, Chromatographia 69 (2009) 1055.
[9] S. Hillaert, W. Van den Bossche, J. Pharm. Biomed. Anal. 31 (2003) 329.
[10] E. Satana, S. Altinay, N.G. Goger, S.A. Ozkan, Z. Senturk, J. Pharm. Biomed. Anal. 25 (2001) 1009.
[11] R. Jain, C.L. Jain, Indian Drugs 28 (1991) 380.
[12] J. Kirschbaum, S. Perlman, J. Pharm. Sci. 73 (1984) 686.
[13] P.K. Owens, L.A. Svensson, J. Vessman, J. Pharm. Biomed. Anal. 25 (2001) 453.
[14] T. Huang, Z. He, B. Yang, L. Shao, X. Zheng, G. Duan, J. Pharm. Biomed. Anal. 41 (2006) 644.
[15] I. Panderi, M. Parissi-Poulou, Int. J. Pharm. 86 (1992) 99.
[16] C.V.N. Prasad, C. Parihar, K. Sunil, P. Parimoo, J. Pharm. Biomed. Anal. 17 (1998) 877.
[17] K. Murat, E. Nevin, J. Pharm. Biomed. Anal. 19 (1999) 477.
[18] C.F.F. Monica, M.C. Patricia, S.K. Teodoro, J. Pharm. Biomed. Anal. 34 (2004) 305.
[19] S. Agatonovic-kustrin, M. Zecevic, L. Zivanovic, I.G. Tucker, Anal. Chim. Acta 364 (1998) 265.
[20] F. Croo, W.V. Bossche, P. Moerloose, Chromatographia 20 (1985) 477.
[21] M.E. Martın, O.M. Hernandez, A.I. Jimenez, J.J. Arias, F. Jimenez, Anal. Chim. Acta 381 (1999) 247.
[22] F.A. El-Yazbi, H.H. Hammud, G.M. Sonji, Int. J. Appl. Chem. 3 (2007) 1.
[23] I. Sperlingova, L. Dabrowska, V. Stransky, M. Tichy, Anal. Bioanal. Chem. 378 (2004) 536.
[24] N.R. Herbert, I. Chappell, J. Wang, S. Anderson, M. Jacyno, W. Luo, Grace Davison Discovery Sciences, 2009, www.discoverysciences.com.
[25] L.R. Snyder, J.W. Dolan, P.W. Carr, Anal. Chem. 79 (2007) 3252.
[26] N.N. Atia, P. York, B.J. Clark, J. Sep. Sci. 32 (2009) 2732.
[27] H. Ibrahim, A. Boyer, J. Bouajila, F. Couderc, F. Nepveu, J. Chromatogr. B 857 (2007) 59.
[28] Department of Health and Human Services, Food and Drug Administration, Guidance for Industry on Bioanalytical Method Validation, Fed. Regist. 66 (2001) 28526.
[29] C. Kaushal, B. Srivastava, J. Chem. Pharm. Res. 2 (2010) 519.
[30] J. Nawrocki, J. Chromatogr. A 779 (1997) 29.
[31] E.G.C. Clarke, A.C. Moffat, Clark's Isolation and Identification of Drugs in Pharmaceuticals Body Fluids, and Post-mortem Material, second ed., The Pharmaceutical Press, London, 1986, p. 1048.
[32] United States Pharmacopeia XXX and NF XXVI, US Pharmacopeial Convention INC.,Twinbrook Parkway, Rockville, MD, 2007, pp. 1374, 1611, 2288, and 3445.