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Resolution of overlapped spectra for the determination of ternary mixture using different and modified spectrophotometric methods

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ABSTRACT

Four new spectrophotometric methods were developed, applied to resolve the overlapped spectra of a ternary mixture of [aliskiren hemifumarate (ALS)–amlodipine besylate (AM)–hydrochlorothiazide (HCT)] and to determine the three drugs in pure form and in combined dosage form. Method A depends on simultaneous determination of ALS, AM and HCT using principal component regression and partial least squares chemometric methods. In Method B, a modified isosbestic spectrophotometric method was applied for the determination of the total concentration of ALS and HCT by measuring the absorbance at 274.5 nm (isosbestic point, A_{iso}). On the other hand, the concentration of HCT in ternary mixture with ALS and AM could be calculated without interference using first derivative spectrophotometric method by measuring the amplitude at 279 nm (zero crossing of ALS and zero value of AM). Thus, the content of ALS was calculated by subtraction. Method C, double divisor first derivative ratio spectrophotometry (double divisor ^{1}DD method), was based on that for the determination of one drug, the ratio spectra were obtained by dividing the absorption spectra of its different concentrations by the sum of the absorption spectra of the other two drugs as a double divisor. The first derivative of the obtained ratio spectra were then recorded using the appropriate smoothing factor. The amplitudes at 291 nm, 380 nm and 274.5 nm were selected for the determination of ALS, AM and HCT in their ternary mixture, respectively. Method D was based on mean centering of ratio spectra. The mean centered values at 287, 295.5 and 269 nm were recorded and used for the determination of ALS, AM and HCT, respectively. The developed methods were validated according to ICH guidelines and proved to be accurate, precise and selective. Satisfactory results were obtained by applying the proposed methods to the analysis of pharmaceutical dosage form.

1. Introduction

The most important strategies for reducing the risk of cardiovascular diseases are the effective control of blood pressure [1,2]. Combination therapy is presented as an effective therapeutic approach for hypertension treatment. Two or more drugs, belonging to different classes of antihypertensives, are combined together in order to maximize the antihypertensive effect, minimize the opposing compensatory effect to a certain drug and minimize the adverse effects by allowing smaller doses of each drug in the combination therapy [2,3]. A recent single pill combination of aliskiren (a novel orally active direct renin inhibitor), amlodipine (calcium channel blocker) and hydrochlorothiazide (diuretic) was formulated [4].

Aliskiren belongs to the new class of nonpeptide renin inhibitors which prevents the conversion of angiotensinogen into angiotensin I and therefore inhibits the production of angiotensin II and aldosterone. It is chemically designated as (2S,4S,5S,7S)-5-amino-*N*-(2-carbamoyl-2-methylpropyl)-4-hydroxy-2-isopropyl-7-[4-methoxy-3-(3′-methoxypropoxy)benzyl]-8-methylnonanamide hemifumarate) (Fig. 1a) [5,6]. Amlodipine besylate, 3-ethyl 5-methyl(4*RS*)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-di-carboxylate benzenesulphonate (Fig. 1b), is a calcium channel blocker with greater affinity for vascular calcium channels than for calcium channels in the heart [2,7]. Hydrochlorothiazide, 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide, (Fig. 1c), is a thiazide diuretic that lowers blood pressure initially by increasing sodium and water excretion [8].

ALS is not yet official in any of the pharmacopoeias. On the other hand, AM was determined in the USP [9] and the BP [10] by HPLC method, using either buffer (pH 3): methanol: acetonitrile (50:35:15, v/v/v) or ammonium acetate: methanol (30:70, v/v) as a mobile phase, respectively. UV detection was carried out at 237 nm.

For HCT, the USP [9] reported an HPLC method for the determination of HCT in bulk using a mobile phase of different mixtures of solution A [acetonitrile: methanol (3:1, v/v)] and solution B [anhydrous formic acid in water] on C_{18} column at a flow rate of 1 ml min^{-1}. UV detection was carried out at 275 nm. Another HPLC method was mentioned for HCT determination in pharmaceutical dosage form using a mobile

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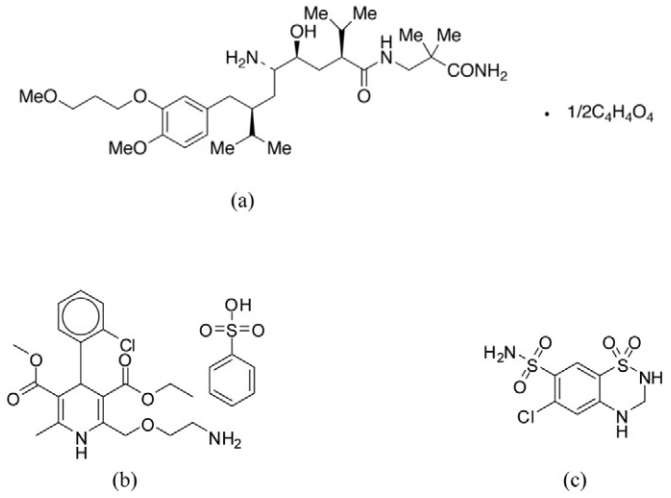

Fig. 1. Chemical structures of ALS (a), AM (b) and HCT (c).

Table 1
The concentrations of different mixtures of ALS, AM and HCT used in the training set.

Mix no.	ALS (μg ml^{-1})	AM (μg ml^{-1})	HCT (μg ml^{-1})
1	60	1.8	4.50
2	60	2.0	5.00
3	60	2.2	5.50
4	150	5.5	11.00
5	150	5.0	12.50
6	150	4.5	14.00
7	270	9.9	20.25
8	270	9.0	22.50
9	270	8.1	24.75

phase consisting of phosphate buffer (0.1 M, pH 3): acetonitrile (9:1, v/v) on a C_{18} column. UV detection was carried out at 254 nm. In the BP [10], HCT was determined potentiometrically in dimethyl sulphoxide using 0.1 M tetrabutylammonium hydroxide in 2-propanol as titrant.

Literature survey reveals few methods for the determination of ALS, AM and HCT in ternary mixture. A capillary electrophoresis method was reported using a running buffer composed of phosphate buffer (40 mM, pH 6). UV detection was carried out at 245 nm [11]. Few HPLC methods with UV detection were described for the simultaneous determination of the mentioned drugs in their ternary mixture [12–16].

The main problem of spectrophotometric multicomponent analysis is the simultaneous determination of two or more compounds in the same mixtures without preliminary separation. No spectrophotometric methods were reported for resolving ternary mixture of ALS, AM and HCT. Therefore the plan of the work comprises the development of new spectrophotometric methods for the resolution of the overlapped spectra of the three drugs in ternary mixture. In addition, determination of the studied drugs in combined pharmaceutical formulation and validation of the developed methods.

Principal component regression (PCR) and partial least squares (PLS) chemometric methods were successfully applied.

Isosbestic method was previously used for the determination of binary mixture of two drugs of equal concentrations. Searching the literature, no isosbestic methods were reported for the determination of two drugs, which are present in unequal concentration in binary mixture. Therefore, a modification in the isosbestic method was introduced in order to determine ALS and HCT, which are present in a ratio of (12:1) in their ternary mixture with AM.

Derivative ratio spectrophotometric method applied for the analysis of ternary mixture is based on measuring the amplitude at the zero-crossing points in the derivative spectrum of the ratio spectra [17]. While double divisor ^{1}DD method is based on the use of double divisor and on measurement at either the maximum or minimum wavelengths [18]. It represents the best solution in case of absence of zero-crossing point.

On the other hand, mean centering of ratio spectra method is a recently developed method that eliminates the derivative steps and therefore, enhance the signal-to-noise ratio [19].

In addition, the theoretical background of the modified methods and recent methods are presented.

2. Experimental

2.1. Instruments

Schimadzu Ultraviolet/Visible recording spectrophotometer 1600/ Japan connected to an IBM compatible computer and supported with UV Probe software version 2.21 was used for chemometric methods. A Jenway 6800 double beam Ultraviolet/Visible spectrophotometer, (UK), connected to an IBM compatible computer with 1 cm quartz cell and supported with Jenway flight deck software was used for the other spectrophotometric methods. Matlab™ software, version 7 with Toolbox 2.0 was used for chemometric and mean centering of ratio spectra methods.

2.2. Materials and reagents

ALS pure sample was purchased from Wuhan Sunrise Technology Development Company, Wuhan, China. Its purity was checked and was found to be 99.54 ± 0.642 [20]. Amlodipine besylate was kindly provided by AlphaChem Advanced Pharmaceutical Industries SAE (ACAPI), Cairo, Egypt. Its purity was checked by a first derivative spectrophotometric method [21] and found to be 100.37 ± 0.954. Hydrochlorothiazide was supplied by Eva Pharma Company (Giza,

Table 2
Different amounts of ALS, AM and HCT mixed to prepare laboratory mixtures for isosbestic and ^{1}D methods, double divisor derivative ratio and mean centering of ratio spectra methods.

Mix no.	Isosbestic and ^{1}D methods			Mix no.	Double divisor derivative ratio method			Mix no.	Mean centering of ratio spectra method		
	HCT working solution (A)	AM working solution (A)	ALS stock solution		ALS stock solution	AM working solution (A)	HCT working solution (A)		ALS stock solution	AM working solution (A)	HCT working solution (A)
1	37.5 μg	15 μg	450 μg	1	450 μg	15 μg	37.5 μg	1	450 μg	15 μg	37.5 μg
2	95.0 μg	38 μg	1140 μg	2	1050 μg	35 μg	87.5 μg	2	750 μg	25 μg	62.5 μg
3	112.5 μg	45 μg	1350 μg	3	1350 μg	45 μg	112.5 μg	3	1350 μg	45 μg	112.5 μg
4	145.0 μg	58 μg	1740 μg	4	1950 μg	65 μg	162.5 μg	4	1740 μg	58 μg	145.0 μg
5	162.5 μg	65 μg	1950 μg	Mix no.	ALS stock solution	AM working solution (B)	HCT working solution (B)	5	1950 μg	65 μg	162.5 μg
Mix no.	HCT working solution (B)	AM working solution (B)	ALS stock solution	5	2100 μg	70 μg	175 μg	Mix no.	ALS stock solution	AM working solution (B)	HCT working solution (B)
6	212.5 μg	85 μg	2550 μg	6	2550 μg	85 μg	212.5 μg	6	2550 μg	85 μg	212.5 μg