Determination of epinephrine in pharmaceutical dosage using hydrophilic interaction chromatography with ICP-AES detection

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Abstract
Hydrophilic interaction chromatography (HILIC) coupled online to ICP-AES was used to investigate the retention characteristics of epinephrine. ZIC-HILIC column with sulfoalkyl-betaine groups on polystyrene-divinylbenzene surface. The zwitter ionic stationary phase can separate the epinephrine-V(V) complex by HILIC-ICP-AES.

The separation mechanism is according to HILIC and ion exchange for the epinephrine. A calibration graph of epinephrine was established for HILIC column and it was found that the range concentration is 20-800 ng.ml^{-1}, RSD% is 0.66-1.55 and LOD is 5.7 ng.ml^{-1}.

Keywords: Epinephrine, Hydrophilic interaction chromatography, ZIC, pharmaceuticals, Zwitter ion chromatography.

Introduction

Epinephrine is *R*)-1-(3,4-di-hydroxy-phenyl)-2-methyl amino ethanol (Figure 1). Epinephrine is used to stimulate heartbeat and to treat bronchial asthma, bronchitis, and emphysema, as well as in the treatment of the glaucoma and eye disease[1,2].

Rasheed et al[3,4] developed spectrophotometric methods for determination epinephrine using vanadium and praseodymium as chelating agents. There are many chromatographic methods for the determination of epinephrine[5-11]. Our objective is to investigate the separation mechanism and retention behavior of epinephrine-V(V) complex.

Rasheed et al[12] and seubert et al[13] suggested a method for analysis of two pharmaceuticals as complexes: desferrioxamine and trifluoperazine using HILIC columns by ICP-OES. They have found the separation of the DFOM as a complex with Ce^{+4} is generally driven by a cation exchange mechanism. This is a behavior never before noticed using zwitter ionic stationary phase. As for the TFPH, the separation of the TFPH as complexes with palladium, vanadium and platinum ions is divided based on cation exchange interactions and electrostatic interaction. Our another objective is to introduce a method for the analysis of epinephrine in pharmaceutical formulations.

Material and Methods

The retention behavior of the epinephrine-V (V) complex, ICP-AES Spectroflame P (Germany) as detectors at λ= 311.071 nm (V) online coupling with IC (Metrohm). ZIC-HILIC column were used for the epinephrine-V (V) complex separation. Grafted 4-vinylbenzyldimethylammonio pentanesulfonate[12-14]. $NaVO_3$, acetic acid, NaOAc and epinephrine were obtained from BDH. ACN was obtained from Merck. Thirteen ampoules for the commercial companies were collected, 1 mg of epinephrine was moved into 25 mL and diluted to the mark with water and, therefore, the epinephrine sample was filtered by the filter (0.22 μm).

Results and Discussion

Separation of epinephrine-V(V) complex: Epinephrine was chosen as test pharmaceutical as it reacts with vanadium to form stable complex. The epinephrine-V(V) complex separation is examined using 80% NaOAc/HAc (45 mmol / pH 5) buffer mobile phase with 20% ACN content. The epinephrine-V(V) complex stoichiometry was 1:2 for epinephrine-V(V). The chromatogram of epinephrine-V(V) complex is shown in figure 2. The influence of pH and eluent concentration of epinephrine-V(V) complex must give a vision about the property of the ZIC-HILIC exchanger and thus about the separation mechanism.

The effect of eluent concetration: To study the influence of eluent concentration, the concentration of the NaOAc/HAc buffer should be varied (30 to 70 mmol) while controlling ACN fraction and the eluent pH at constant (20% ACN, 5.5 pH). The retention factor of epinephrine-V(V) complex decreased with an increasing NaOAc/HAc concentration. Figure 3 exhibits the slope for changing eluent NaOAc/HAc concentration similar to the slope for cation exchange mechanism[15]. Consequently, the retention behavior and separation mechanism of the epinephrine-V(V) complex contributed greatly for the ion-exchange interactions with the ZIC-HILIC column[16].

The effect of mobile phase pH: For the separation mechanism of epinephrine-V(V) complex, the pH of the NaOAc/HAc buffer should be varied from 3 to 6 while controlling ACN fraction and the eluent concentration at constant (20% ACN, 5.5). The retention factor of epinephrine-V(V) complex decreased with an increasing NaOAc/HAc pH (Figure 4). The reason of this behaviour is due to the p*k*a ~8.91 of epinephrine and that means the

protonation of the epinephrine-V(V) complex in the pH range between 3 to 6[17].

Method Validation

Linearity and calibration of epinephrine: A calibration graph of epinephrine as epinephrine-V (V) complex exhibits the range concentration (20-800 ng.ml^{-1}) (Figure 5).The direct calibration graph for the direct determination of epinephrine-V(V) was constructed and the statistical results are illustrated in table 1.The same-day and the day-to-day accuracy and precision were examined calculating by recovery % and RSD %, respectively. The low relative standard deviation values and the high recovery values refer that the suggested method is precise (Table 2).

Determination of epinephrine in sample of pharmaceutical preparation: Five types of pharmaceutical formulations containing epinephrine (ampoule) have been studied and they gave a good precision and accuracy as in table 3. The suggested method was also applied successfully on five types of injection. For analyzing the success and the competence of the ZIC-HILIC suggested technique, the results obtained were in contrast to those obtained by regular USP (united state pharmacopeia) technique [18]. The outcomes obtained by the suggested methods table 4 were statistically compared, making use of F-test and t-test at 95% confidence level[19]. The calculated F- and t- values did not go over the theoretical values, which suggest that there is no great distinction between both techniques in precision and accuracy in the analysis of epinephrine in pharmaceutical dosages.

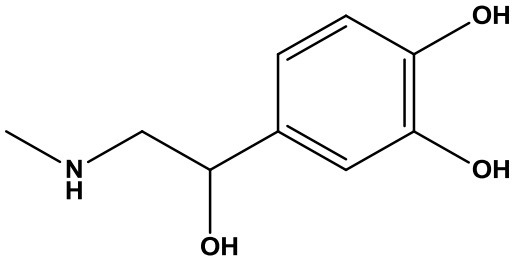

Figure 1: Structure formulae of epinephrine

Table 1
Representative statistical results for the analysis epinephrine

Parameter	Epinephrine
Range of concentration (ng.ml^{-1})	20-800
Detection limit (ng.ml^{-1})	5.7
Regression line	y = -3.1109 + 1.4655*x
Correlation coefficient (r)	0.9993

Table 2
Precision and accuracy of the suggested method-epinephrine.

Same-Day Analysis n=5					Day-to-Day Analysis n=5			
Epine. Taken ng.mL^{-1}	Epine. Found ng.mL^{-1}	%Rec.	% Erel.	%RSD	Epine. Found ng.mL^{-1}	%Rec.	% Erel.	%RSD
65	64.75	99.61	-0.38	1.55	65.16	100.24	0.24	1.21
130	130.22	100.17	0.17	0.66	130.33	100.25	0.25	0.66

Table 3
The suggested method for determination of epinephrine as epinephrine-V(V) in pharmaceutical formulations.

Name of pharm.	Manufacturer	Stated conc. (mg)	Found direct calb. (mg)	Rec. %	RSD% n=5	E_{rel} %
Epinephrine (Ampoule)	Hospira-USA	1000	997.22	99.72	0.68	-0.28
Epinephrine (Ampoule)	JHP Pharmaceuticals-USA	1000	1010.35	101.03	1.12	1.03
Epinephrine (Ampoule)	Misr Co. for Pharma Ind.- Egypt	1000	989.66	98.96	0.45	-1.03
Epinephrine (Ampoule)	Lab. Renaudin-France	1000	994.76	99.47	1.02	-0.52
Epinephrine (Ampoule)	Hameln-Germany	1000	1005.78	100.57	0.73	0.57

Table 4
The comparison of the suggested method with official method to determination epinephrine-V(V) using t- and F-statistical tests

Name of pharmaceutical	Suggested method	official method	t-test (theor.)	F-test (theor.)
	Rec.% *	Rec.% *		
Epinephrine (Ampoule) Hospira-USA	99.72	100.13	0.7159 (2.306)	5.4130 (9.605)
Epinephrine (Ampoule) Hameln-Germany	100.57	100.67		

*Average of five determinations

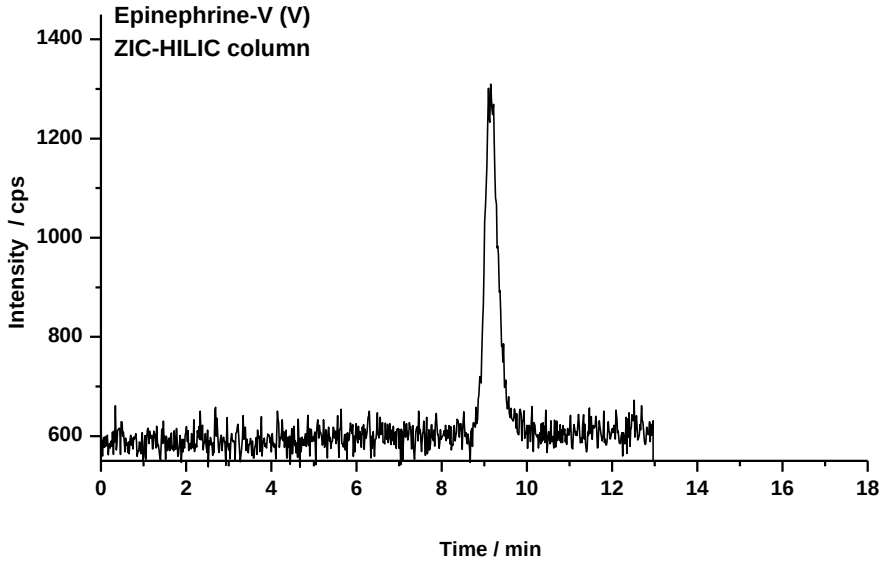

Figure 2: Chromatograms for the separations of epinephrine-V(V).

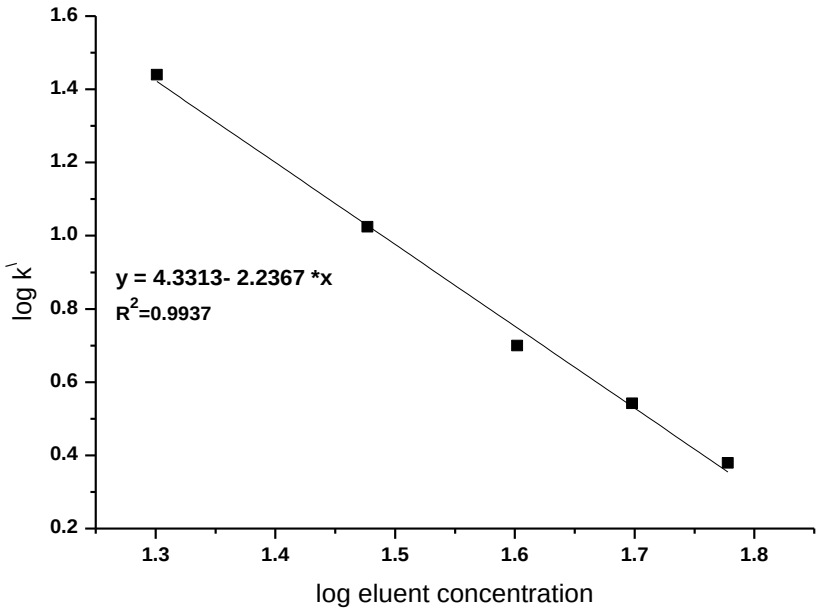

Figure 3: Effect of buffer strength on the epinephrine-V(V) complex retention using ZIC-HILIC column

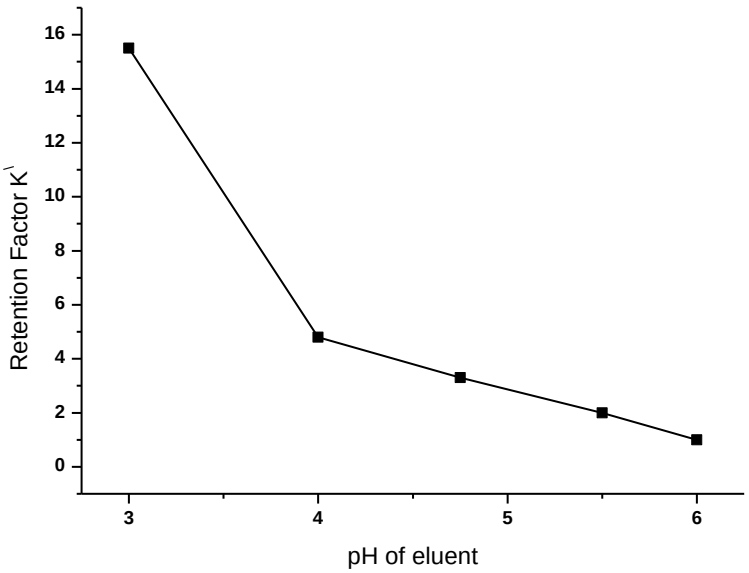

Figure 4: Effect of eluent pH on the epinephrine-V(V) complex retention using ZIC-HILIC column

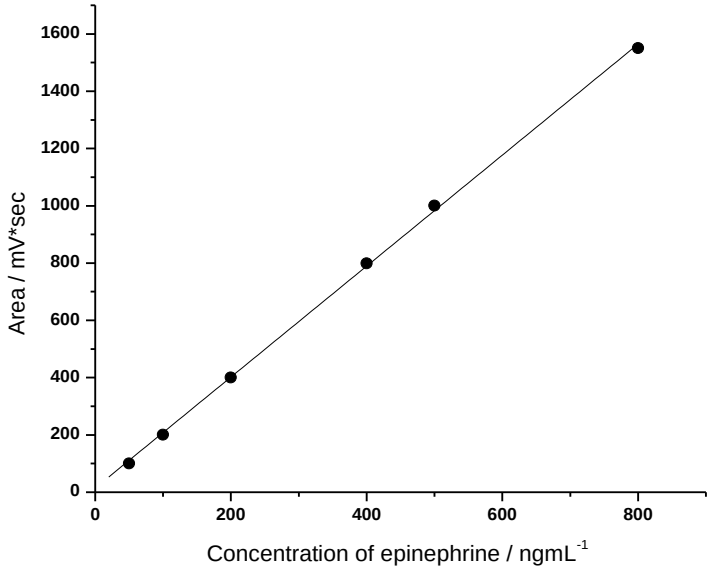

Figure 5: Calibration curve for epinephrine using ZIC-HILIC column.

Conclusion

Hydrophilic interaction chromatography allowed the separation mechanism of epinephrine-V(V). The present study involves the development of hydrophilic interaction chromatography method for the analysis of epinephrine dosages. The experimental data exhibited that both ion exchange and hydrophilic interaction chromatography behaviors are active as retention mechanism. The developed method was successfully applied to the analysis of epinephrine in pharmaceutical dosages. Statistical data analysis, F-Test and T- exhibited that there is no great impact on precision and accuracy between the suggested methods as well as the official method.

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