
بسم الله الرحمن الرحيم

Validated stability - indicating capillary electrophoresis method for the separation and determination of fixed dose combination of carvedilol and hydrochlorothiazide in tablets

Maha A. Sultan, Nora Z.Alzoman, Hadir M. Maher , Mona M. Alshehree, Ileana V. Olah

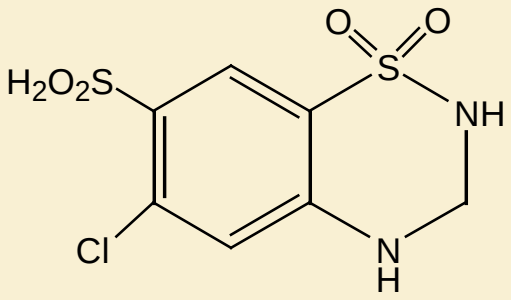
Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11495, P.O. Box 22452, Saudi Arabia

Carvedilol is a non-cardio selective β-blocker It also has vasodilating properties. It is used in the management of hypertension and angina pectoris.

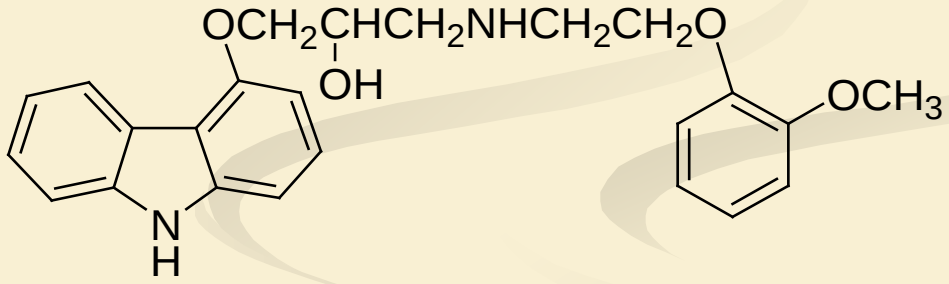
Hydrochlorothiazide is a diuretic of the class of benzothiadiazines widely used in antihypertensive pharmaceutical preparations which decrease active sodium reabsorption and reduce peripheral vascular resistance.

Combined therapy of CRV and HCT had a significantly greater blood pressure reduction than with the same dosage of CRV alone. Also, a combination of CRV and HCT are used often to treat heart trouble in the clinic.

Chemical structure of carvedilol and hydrochlorothiazide



hydrochlorothiazide



carvedilol

Aim of the work:

- **The objective** of this work was to develop an analytical CE-DAD procedure, which would serve as stability indicating assay method for combination drug product of CRV and HCT.

- **The purpose of stability testing** is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and enables recommendation of storage conditions, retest period, and shelf lives to be established.

- The main aspects of drug product that play an important role in shelf life determination are assay of active drug, and in presence of degradants generated, during the stability study.

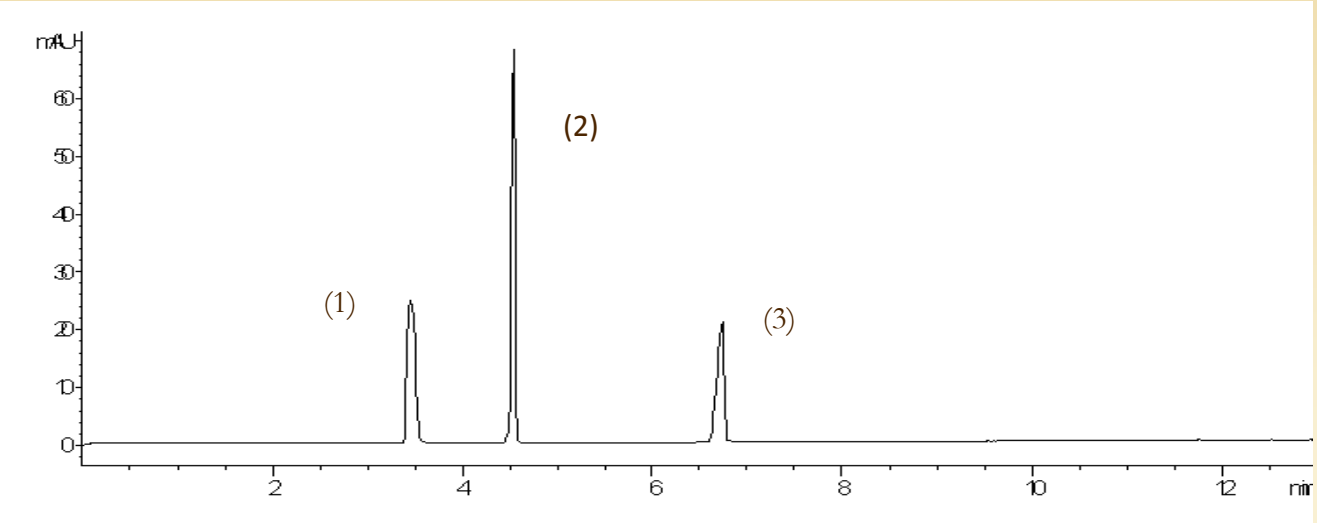
Optimization of Electrophoretic conditions

The employed CE system consisted of an Agilent Capillary Electrophoresis instrument (Agilent Technologies Deutschland, GmbH, Hewlett-Packard- Stress 8, Germany) equipped with a photodiode array detector (DAD) and a data handling system comprised of an IBM personal computer and Agilent Chem Station software.

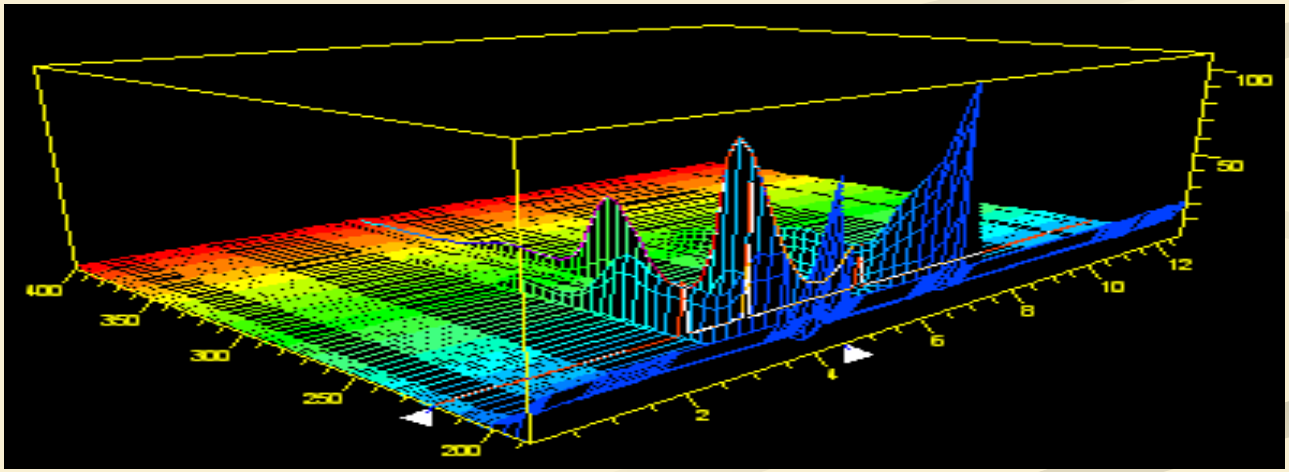
Detection **was performed at 226 nm.**

- ***A deactivated fused silica capillary*** **was obtained from Agilent Technology (Fullerton, CA, USA) had the following dimensions 65 cm total length, 55 cm effective length and 75 µm ID.**
- ***The background electrolyte solution*** **(BGE) consisted of phosphate buffer (12.5 mM, pH 7.4)-methanol in a ratio of 95:5, v/v.**
- ***The temperature*** *of the capillary and the sample was maintained at 24°C.*
- ***pressure*** *at the anodic side at 40 mbar for 10s.*
- ***applying high voltage (****30 KV) to the capillary, with the cathode being at detector end.*

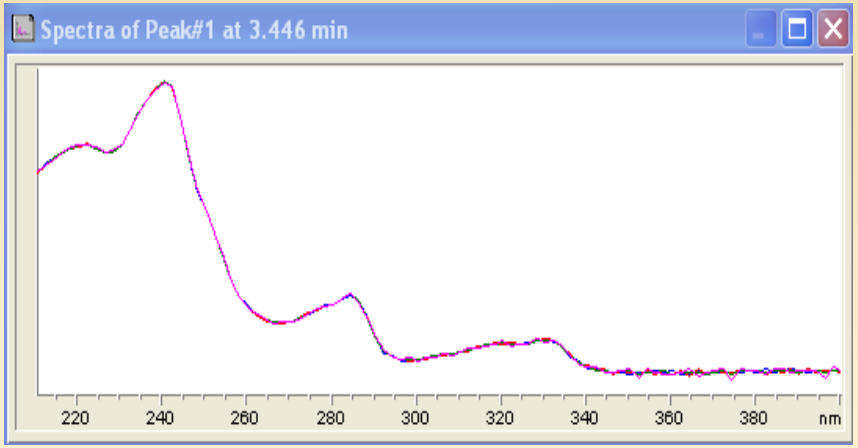
- The capillary was washed between run with deionized water (3min), then equilibrated with the running buffer (5 min), to ensure reproducibility of the assay. Before sample injection, the capillary was conditioned with 0.1 M sodium hydroxide (5min), deionized water (5min) and running buffer electrolyte (10min).



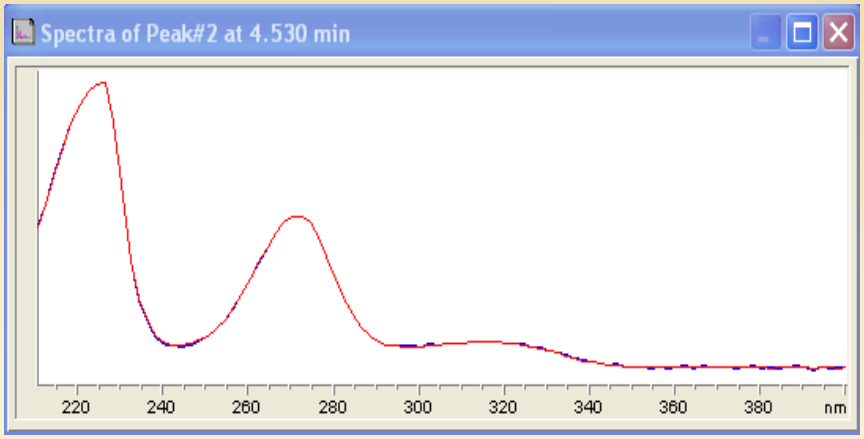
A typical electropherogram of a standard mixture of 80 µg/mL CRV (1), 50 µg/mL HCT (2) and 80 µg/mL ATO, IS (3),



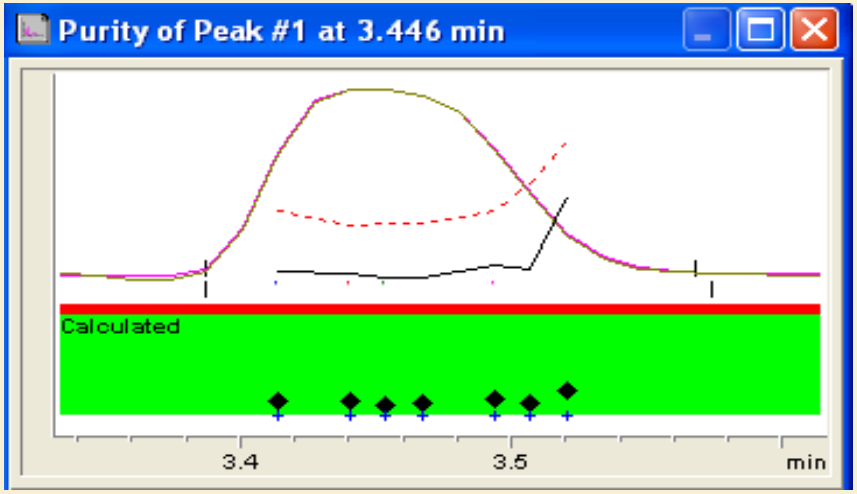
the corresponding 3D plot,

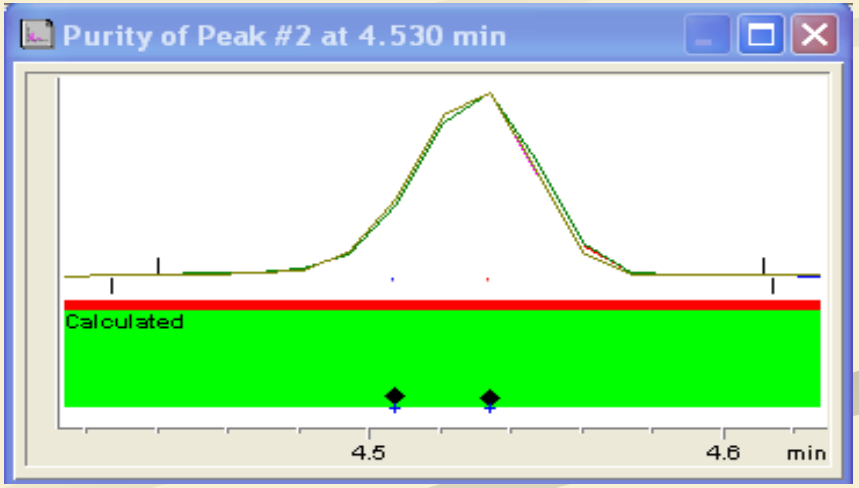


absorption spectra of CRV



absorption spectra of HCT





their corresponding peak purity graphs.

ICH Validation Requirements & Parameters

The proposed CE method was validated in compliance with ICH guidelines.

The following parameters were validated. Atorvastatin (ATO) was used as internal standard for the purpose of quantification of CRV and HCT

ICH

- Specificity
- Linearity
- Range
- Accuracy
- Precision
  - Repeatability
  - Intermediate Precision
- Limit of Detection
- Limit of Quantitation

Performance characteristics of CRV and HCT by the proposed CE method.

	t_m	N	K´	α	R_S	T_f
CRV	3.45	7371	5.90			1.05
				1.37	15.46	
HCT	4.53	55667	8.06			1.10
				1.55	22.67	
ATO	6.75	46656	12.5			1.11

t_m: Migration time, in min.
N :Number of theoretical plates.
K': Capacity factor.
α: Selectivity, between each two successive peaks.
R_s :Resolution, between each two successive peaks.
T_f :Tailing factor.

Effect of various parameters on separation of CRV and HCT

Parameter	Migration time (min)	
	CRV	HCT
pH (phosphate buffer, 12.5 mM, 25° C, 15KV, 0% methanol, 40 mbar)		
2.5	8.50	9.51
4.5	6.83	7.90
5	4.41	5.90
7	3.40	4.61
7.4	3.42	4.59
8.5	3.89	5.12
Buffer concentration,(mM) (phosphate buffer, pH 7.4, 25° C, 15KV, 0% methanol, 40 mbar)		
7.5	3.80	5.25
10	3.85	5.30
12.5	3.49	4.65
15	3.90	5.30
25	5.71	7.63
Organic modifier, (%) (phosphate buffer, pH 7.4, 25° C, 15KV, 40 mbar)		
0	3.61	4.90
5	3.54	4.54
10	4.13	6.56
20	6.45	8.29
25	6.46	8.30
Voltage, (KV) (phosphate buffer, pH 7.4, 25° C, 5% methanol, 40 mbar)		
15	3.53	4.72
20	3.40	4.40
30	3.40	4.61
Pressure, (mbar) (phosphate buffer, pH 7.4, 25° C, 30 KV, 5% methanol)		
40 mbar/ 10 sec.	2.90	3.71
50 mbar/ 10sec.	3.23	3.90
60 mbar / 10sec.	3.44	4.53

Regression and statistical parameters for the determination of CRV and HCT using the proposed method

Parameters	CRV	HCT
Linearity range (µg/mL)	1 – 200	0.2 – 150
Intercept (a)	-0.0110	0.0697
Slope (b)	0.8650	2.0052
Correlation coefficient (r)	0.9995	0.9999
S_a	0.54	0.45
S_b	0.0140	0.00093
$S_{y/x}$	0.074	0.044
LOQ (µg/mL)	0.86	0.20
LOD (µg/mL)	0.26	0.07

S_a = standard deviation of intercept
S_b = standard deviation of the slope
$S_{y/x}$ = standard deviation of the residual

Determination of CRV-HCTZ laboratory-made mixtures using the proposed CE method

Ratio CRV/ HCT	Nominal value, µg/mL		Recovered% ± SD[a]		RSD, %	
	CRV	HCT	CRV	HCT	CRV	HCT
1:1	20	20	99.95 ±1.1	101.0 ±0.9	1.11	0.89
2:1	150	75	101.6 ± 0.5	100.2 ± 0.5	0.49	0.50
3:1	90	30	98.7 ± 0.4	101.3 ± 0.9	0.41	0.89
1:3	40	120	100.0 ± 0.6	101.1 ± 0.7	0.60	0.69

[a] Mean ± SD of five determinations

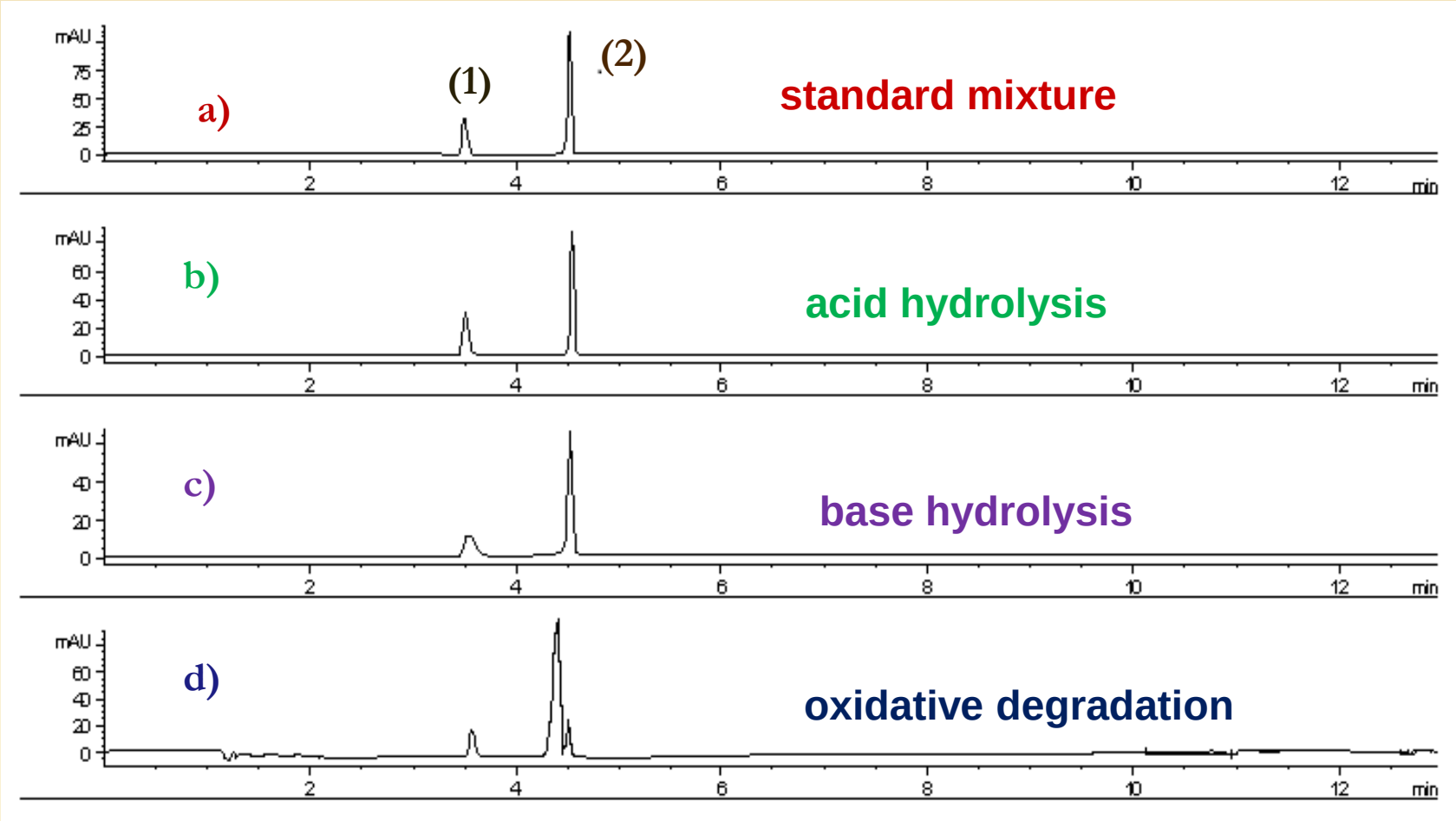
Summary of intra-day (repeatability) and inter-day (intermediate precision) variability data for simultaneous determination of CRV and HCT standards

Compound	Theoretical concentration (µg /mL)	Intra-day measured concentration (µg /mL)[a]		Inter-day measured concentration (µg/ mL)[b]	
		Mean	RSD%	Mean	RSD%
CRV	180	178.92	0.3	179.64	0.5
	130	130.91	1.0	130.55	0.5
	30	30.15	1.2	30.00	0.9
HCT	120	120.72	0.6	120.51	0.6
	60	60.00	0.2	60.33	0.5
	15	15.04	1.2	15.07	0.4

[a] Mean values represent six different samples standards for each concentration

[b] Inter-day precision was determined from six different runs over three successive days.

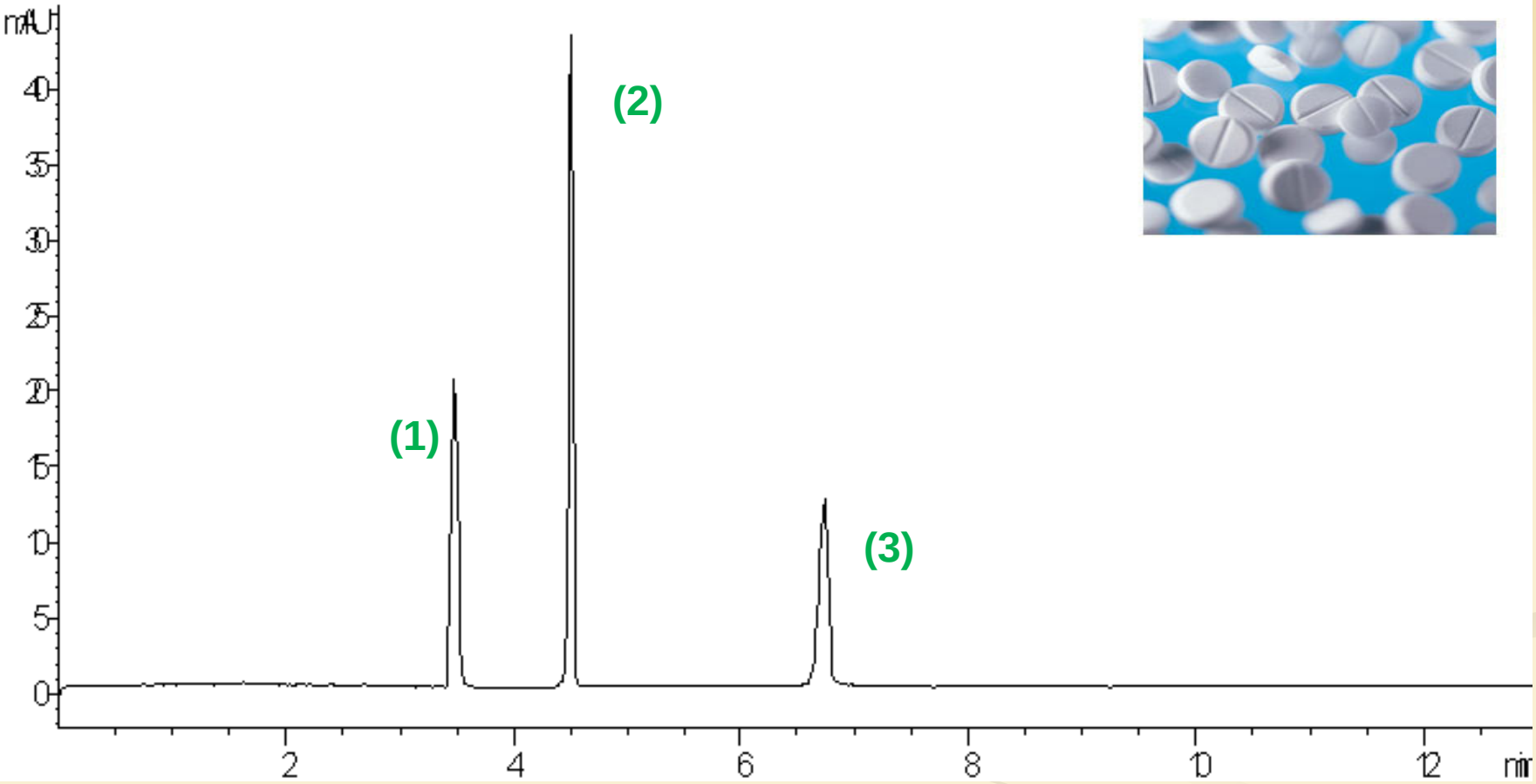
Stress degradation study



A typical electropherogram of a standard mixture containing 100 µg/ml CRV (1) and 100 µg/ml HCT (2), a) and its corresponding acid hydrolysis, b), base hydrolysis, c) and oxidative degradation, d).

Summary of degradation studies

Stress conditions	% recovery	% degradation	Purity factor[a]	Purity threshold
Carvedilol				
Acid hydrolysis(1.0 N HCl, R.T., 5 hr)	97.55	2.45	999.853	993.878
Basic hydrolysis(1.0 N NaOH, R.T., 5 hr)	68.80	31.20	998.937	980.157
Oxidative degradation (30 % v/v H_2O_2, R.T. 1hr)	Not affected		998.886	992.973
Thermal decomposition (at 80°C, 4 hr)	Not affected		999.920	994.789
Photodegradation (direct day light, 24 hr)	Not affected		999.988	998.316
Hydrochlorothiazide				
Acid hydrolysis(1.0 N HCl, R.T., 5 hr)	68.42	31.57	999.939	999.410
Basic hydrolysis(1.0 N NaOH, R.T., 5 hr)	66.80	33.10	999.755	995.987
Oxidative degradation (30 % v/v H_2O_2, R.T. 1hr)	74.10	35.90	999.257	987.198
Thermal decomposition (at 80°C, 4 hr)	Not affected		998.427	991.930
Photodegradation (direct day light, 24 hr)	Not affected		999.964	999.767



A chromatogram of the prepared tablet solution of Co-Dilatrend®

containing 80 µg/mL CRV (1), 40 µg/mL HCT (2) and 80 µg/mL ATO, IS (3).

Statistical analysis of assay results and recovery experiments in commercial tablets

	Proposed CE		Reference,HPLC method		Standard addition	
	CRV	HCT	CRV	HCT	CRV	HCT
Co-Dilatrend tablets, (25mg CRV, 12.5mg HCT)						
Recovery,% ± SD[a]	100.7±0.36	101.3±0.84	100.63±0.43	101.85±0.35	99.5±1.1	101.2±0.6
RSD %	0.36	0.41	0.43	0.34	1.11	0.59
t[b]	0.25	1.24				
F[b]	1.42	5.76				
Co-dilatrol tablets (25 mg CRV, 12.5 mg HCT)						
Recovery,% ± SD[a]	98.29±0.45	98.47±0.62	98.18±0.53	98.94±1.50	98.47±0.62	99.00±0.95
RSD %	0.46	0.63	0.0.54	1.52	0.63	0.96
t[b]	0.0.35	0.65				
F[b]	1.39	5.92				

[a] Mean and RSD% for five determinations

[b] Theoretical values of F and *t* for p = 0.05 and n=5 are 6.39 and 2.31, respectively.

Thank you