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Rapid and Sensitive CZE Method for Quality Control Analysis of Pharmaceuticals Containing Pseudoephedrine, Triprolidine and Paracetamol

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Abstract

A rapid and sensitive CZE (Capillary Zone Electrophoresis) method for pharmaceutical analysis was developed and fully validated. The active compounds: Pseudoephedrine hydrochloride (PSE), Triprolidine hydrochloride (TRI) and Paracetamol (PAR) were separated and quantitatively determined using the tris-borate 30 mM buffer at pH = 9.0 as a Background Electrolyte (BGE). The electrophoretic separation was carried out at 25 kV in an unmodified fused silica capillary of I.D. = 50 µm with a "bubble-cell" for UV detection at 210 nm and 25˚C. The separation was reached in about 3 min. After calibration the method was applied for analysis of three commercially available pharmaceutical preparations. The repeatability (RSD%) of migration time (t_m) was ranging between 0.47% and 0.90% and of peak areas (A) between 0.63% and 3.64%. The Limit of Detection (LOD) values was of 0.19 µg/mL, 0.31 µg/mL and 0.08 µg/mL for respectively PSE, TRI and PAR. The results obtained in this study showed that the proposed method was useful in routinely analysis of pharmaceuticals.

Keywords

Capillary Zone Electrophoresis, Triprolidine, Pseudoephedrine, Paracetamol, Pharmaceutical Analysis, Validation

1. Introduction

The Quality Control in pharmaceutical industry needs new reliable analytical methods able to analyse the active

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compounds present in the produced formulations. The composite tablets containing PSE, TRI, and PAR are commonly used for influenzal disease. Many analytical methods are being developed for determination of these compounds present in various preparations as individual component or in presence of other compounds. The spectrophotometric determination was used for these purposes successfully [1]-[3]. Some authors developed an electrochemical sensor for PAR determination [4]. Two chromatographic methods: TLC and HPLC were optimized and compared for PAR, PSE and cetrizine determination [5]. The HPLC separation result the most frequently technique applied for this purpose [6]-[12]. Only three papers describe the simultaneous HPLC analysis of active ingredients of our interest: PAR, PSE and TRI in pharmaceuticals [13]-[15]. Recently the Capillary Electrophoretic technique reveals very useful in pharmaceutical analysis. CZE separation and determination of PSE, PAR and dextromethorphan in composite tablets is described [16]. The PSE present in the mixture of other seven drugs, was successfully separated and quantitatively determined by CZE method [17]. Other authors proposed [18] the CZE procedure for determination of PAR and codeine in pharmaceuticals. PAR, PSE; dextromethorphan and chlorpheniramine were separated and quantitatively determined in medicinal preparations by flow-injection-CE [19]. Triprolidine and other antihistamines present in pharmaceuticals and serum samples were analysed by CZE [20]. There are no publications about simultaneous CZE analysis of these three active ingredients in pharmaceuticals. The CE technique presents many advantages: rapidity, efficiency, versatility and low reagent consuming. Therefore it seems useful to consider the possibility of develop a capillary electrophoretic method for routine analysis of pharmaceuticals containing PAR, PSE and TRI, as alternative of HPLC.

2. Experimental

2.1. Instrumentation

The apparatus used for the analyses was an Agilent 7100 CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a UV-DAD detector. The uncoated fused silica capillary with extended light path CE ("bubble cell") was from Agilent. For pH measurement a Crison pH/mV meter 506 was used. Detection wavelength was set at 210 nm. The injection conditions were of 2 sec under 80 mbar.

2.2. Chemicals and Materials

All reagents were of analytical grade purity.

The drug standards, Borax (sodium tetraborate decahydrate), Trizma-base (tris-hydroxymethylamino-methane) were purchased from SIGMA-ALDRICH (Steinheim, Germany). Boric acid was from Merck (Darmstad, Germany).

Three commercially available pharmaceutical preparations were analysed: ACTIGRIP and ACTIFED from Johnson & Johnson S.p.A (ITALY) and ACTIFED RHUME from Johnson & Johnson Santé Beauté (FRANCE). For solution filtering the syringe filtres 0.45 µm from (Millex HV, Millipore, MA, USA) were used.

2.3. Buffer, Standard Solutions and Real Samples Preparation

Tetraborate buffer at pH = 9.2 was obtained dissolving the adequate aliquot of sodium tetraborate in distilled water. Tris-borate buffer at Ph = 9.0 was prepared potentiometrically titrating an acqueous solution of tris-base with a saturate solution of boric acid to pH = 9.0.

For the calibration step all standard solutions of analysed drugs were prepared diluting the stock solution (1 mg/mL) to desired concentration. For real samples solutions preparation, twenty tablets were grounded and the quantity of powder corresponding to one tablet (accurately weighted) was dissolved in 50 mL of distilled water. After sonication for 15 min, the solution was filtered through the 0.45 µm syringe filter and diluted to the desired concentration order.

3. Results and Discussion

3.1. Method Development and Optimization

The best experimental conditions for CZE analysis were defined exploring the following parameters: capillary type and dimensions, BGE composition, concentration and pH, applied voltage, injection conditions and detection wavelength.

3.1.1. Capillary Choice

In the preliminary analyses an uncoated silica capillary of 50 µm I.D. was applied obtaining the relatively low sensitivity. Performing the analyses in the capillary with a "bubble cell" detection, the sensitivity increased 3 times. Therefore the extended light path capillary was chosen.

3.1.2. BGE Selection: Composition, Concentration and pH

Three buffers were tested in order to obtain a good resolution of studied analytes in a short time: phosphate, borate and tris-borate. The concentration was ranging between 10 and 40 mM. For the pH optimisation the range between 8.0 and 9.2 were considered. The tested buffer solutions were at hight pH because the greater magnitude of EOF provided shorter analysis time. In the uncoated silica capillary used for analysis, the EOF resulted very high and, consequently, the analysis time short. The symmetric and baseline resolved peaks in a very short time were obtained only in the 30 mM tris-borate BGE at pH = 9.0. These conditions were chosen as optimal. The electropherogram obtained injecting the standard solution of PSE, TRI and PAR in optimal conditions is reported in **Figure 1**.

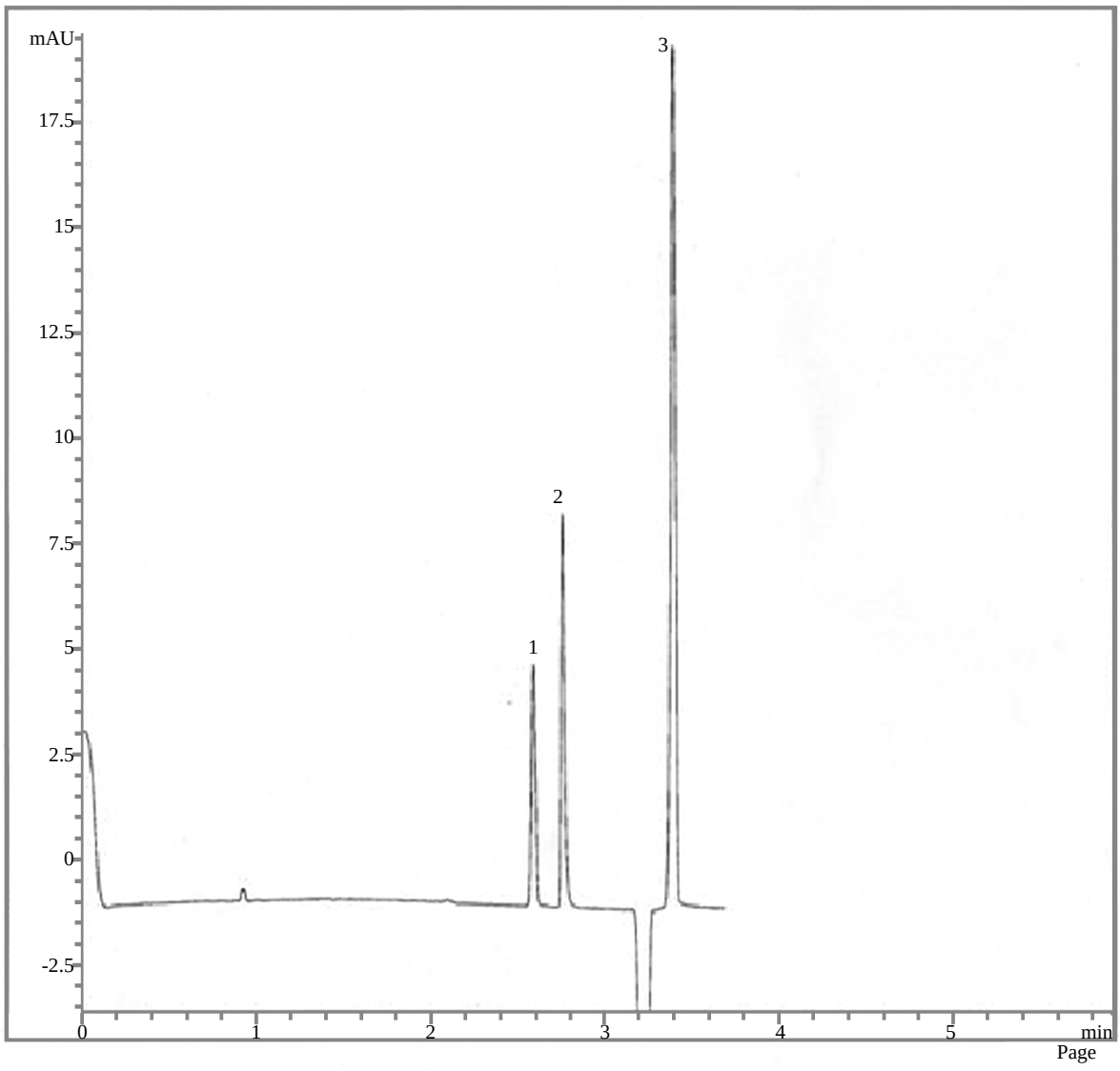

Figure 1. Electropherogram of standard mixture in optimal operational conditions: Tris-borate buffer 30 mM; pH = 9.0; V = 25 kV (i = 1 µA); T = 25˚C; injection at 80 mbar for 2 s; fused silica capillary I.D. = 50 µm, L = 56 cm, l = 48 cm; "bubble cell" detection at 210 nm. Peak identification: 1) PSE, 2) TRI, 3) PAR.

3.1.3. Voltage Optimization

The tested voltage ranging between 10 and 30 kV. Increasing voltage the analysis time decrease and the best compromise at 25 kV was reached. In the conditions assessed of tris-borate 30 mM buffer at pH = 9.0, applying 25 kV generated a very low current: 1 μA. The heat generated during the analysis was very low and, consequently, the repeatability was satisfactory.

3.1.4. Injection Condition

To obtain the symmetric and efficient peaks, the sample zone injected into capillary must be very narrow. Two experimental parameters are responsible on the injected zone: the injection time and pressure. In our study the pressure of 80 mbar applied for 2 sec reveals the best.

3.2. Method Validation

For method validation the ICH guideline was applied [21]. In optimal operational conditions the following parameters were explored: Precision, Specificity, Linearity Range, LOD and Accuracy (Recoveries).

3.2.1. Precision

The run-to-run repeatability of migration time and peak area were evaluated for 7 consecutive injections of the standard and real sample solutions. For the standard solutions three concentration levels (low, intermediate and high) were explored.

The corresponding RDS % values are reported in **Table 1**.

3.2.2. Specificity

The specificity of the CZE method is demonstrated by complete separation of the three analysed active compounds in both the standard solutions and in real samples in presence of excipients. No interferences were observed. The obtained peaks are symmetric, efficient and baseline resolved. For specificity confirming, the UV-spectra of standards and real samples were overlayed.

3.2.3. Calibration Range and Detection Limits (LOD)

For the quantitative analysis the external standard method was applied five standard solutions containing three studied analytes were injected in triplicate in the optimal analysis conditions. The calibration ranges were established taking into account the nominal quantities of active compounds in the analysed pharmaceutical preparations.

For calibration lines obtaining, the peak area was reported versus standard solutions concentration.

The LOD was evaluated for signal-to-noise ratio S/N = 3.

The linearity ranges, regression equations, correlation coefficient and detection limit (LOD) of the active compounds are collected in **Table 2**.

3.2.4. Analysis of Real Samples

Three types of commercially available composite tablets were analysed: Actigrip, Actifed Rhum and Actifed.

A typical electropherogram is shown in **Figure 2**.

No interferences of excipients were observed in real samples analyses. The obtained results are collected in **Table 3**.

Table 1. Repeatability (RSD %) of both the migration time (t_m) and the peak area (A) at low, intermediate and high concentration level of all considered analytes under optimized experimental conditions.

Analyte	RSD% standard solutions				RSD% real samples	
	t_m	A			t_m	A
		Low conc.	Intermediate conc.	High conc.		
PSE	0.47	0.77	0.63	1.65	0.67	3.64
TRI	0.47	3.24	1.96	2.96	0.90	3.36
PAR	0.52	1.63	0.67	2.38	0.77	1.35

Table 2. Calibration data and LOD.

Analyte	Calibration Range (µg/mL)	Equation	R^2	LOD (µg/mL)
PSE	3 - 40	Y = 0.3558x − 0.1124	0.9997	0.19
TRI	10 - 40	Y = 0.4479x − 0.8224	0.9995	0.31
PAR	5 - 70	Y = 0.6727x − 0.7108	0.9985	0.08

Table 3. Analysis of real samples.

Product	Actigrip mg/tablet		Actifed Rhume mg/tablet		Actifed mg/tablet	
Analyte	Found	Declared	Found	Declared	Found	Declared
PSE	50.13	60.00	51.61	60.00	63.35	60.00
TRI	2.80	2.50	2.32	2.50	2.39	2.50
PAR	281.40	300.00	447.04	500.00	-	-

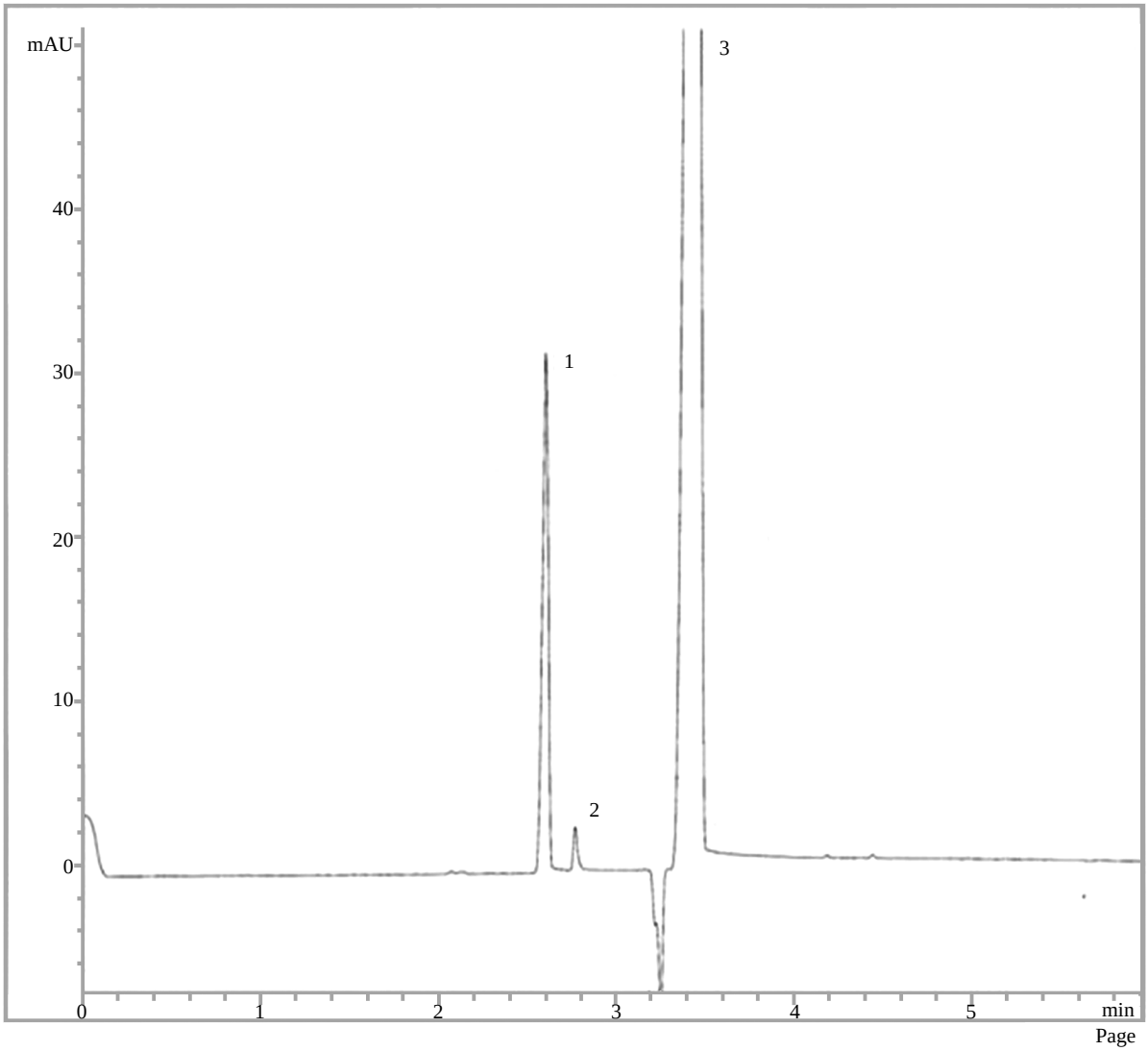

Figure 2. Electropherogram of ACTIGRIP preparation. The operational conditions and peak identification as in **Figure 1**.

Table 4. Recoveries (%).

Analyte	Actigrip	Actifed Rhume	Actifed
PSE	101.2	104.0	97.8
TRI	89.7	92.2	108.4
PAR	95.1	96.2	-

3.2.5. Accuracy (Recoveries)

For accuracy evaluation, the recovery assays on the pharmaceutical products were performed.

First an aliquot of real sample was analysed. In the second step an identical aliquot of real sample was fortified with a known standard amount. After homogenization the sample undergo analysis.

Comparing the concentrations of analytes without fortification and after fortification, the recoveries were calculated. The resulting data are collected in **Table 4**.

4. Conclusions

The developed CZE method proposes a very simple and rapid procedure for analysis of commercially available pharmaceutical tablets containing PAR, PSE and TRI. For the preparative step, a simple dissolution of samples in distilled water and filtration was realised. In the optimized experimental conditions, the studied analytes were completely resolved in about 3 min. No interferences of the excipients were revealed.

Application of proposed method for analysis of three pharmaceutical preparations and subsequently validation makes it a valid alternative for routine analysis in pharmaceutical Quality Control processes. This CE method, with respect to the HPLC, is less time and solvent consuming and, consequently, lowers costs and environmental impact of analysis.

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