Determination of Rimantadine in Pharmaceutical Preparations by Capillary Zone Electrophoresis with Indirect Detection or after Derivatization

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Key Words

Capillary zone electrophoresis
Rimantadine
Indirect detection
Dissolution testing

Summary

Rimantadine is synthetic analog of amantadine; both are antiviral agents used for prophylaxis and treatment of influenza A. A capillary zone electrophoretic (CZE) procedure for the determination of rimantadine has been developed. As the direct determination of rimantadine is poorly sensitive because the compound is almost transparent in the UV/Vis range, several indirect methods were studied. Two were found to be the particularly useful: (a) indirect detection using 5 mM 4-methylbenzylamine in 1:4 methanol-water as absorbing background electrolyte, with detection at 210 nm, and (b) derivatization of rimantadine with 1,2-naphthoquinone-4-sulfonic acid in alkaline medium and subsequent determination of the derivative by CZE (40 mM tetraborate, pH 9.2, detection at 280 nm). Uncoated capillary tubing, 44 cm length × 75 µm i.d., was used for both determinations. The detection limits were 0.1 and 2 ppm for methods a and b, respectively. The methods were used to determine rimantadine in pharmaceutical products and for dissolution testing of Flumadin® tablets.

Introduction

Rimantadine hydrochloride (∝-methyl-1-adamantamethylamine hydrochloride) and amantadine hydrochloride (1-adamantanamine) are antiviral agents used for prophylaxis and treatment of influenza A virus. The amantadine analog rimantadine has greater activity [1, 2] and is considered to be better tolerated than amantadine by humans [3–5].

Analytical methods for the determination of rimantadine in plasma and urine are well documented [6]; they usually involve laborious extraction and derivatization before analysis. Gas chromatography coupled with mass spectrometric detection (GC-MS) is the most common method of determination of the final derivatives. The enantiomers of rimantadine and the compound's hydroxylated metabolites have been determined by GC-MS using stable isotope dilution, selective ion monitoring and methane negative-ion chemical ionization [7, 8], methods which are again rather laborious and time-consuming. Derivatization necessitates working in non-aqueous media and so is not suitable for dissolution testing, because of the large number of samples involved, for example. For this reason we have focused on the development of a method suitable for determination of rimantadine in pharmaceutical preparations, e.g. tablets, and for analysis of dissolution test solutions by capillary zone electrophoresis (CZE) using UV/Vis detection. Because rimantadine is almost transparent in the UV/Vis range the direct determination of the compound is poorly sensitive and several indirect methods were studied, including indirect detection using an absorbing background electrolyte and/or derivatization of the rimantadine amino group (before analysis). The determination of rimantadine by capillary isotachophoresis based on indirect conductivity detection of the hydrochloride has been already reported [9] but this method does not determine the active compound. Therefore, in this work we have studied the possibility of using CZE for the determination of rimantadine. Preliminary results have been reported elsewhere [10].

Experimental

Chemicals

Rimantadine was purchased from Aldrich (Steinheim, Germany), 4-methylbenzylamine from Fluka (Buchs, Switzerland), and 1,2-naphthoquinone-4-sulfonic acid of high-purity grade from Lachema (Brno, Czech Republic). The other reagents, methanol, sodium tetrabo-

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rate, boric acid, Tris, etc., were analytical grade from Lachema. The thrice-distilled water used was produced in a commercial quartz apparatus from Heraeus (Hanau, Germany). Standard pH buffers were from the Institute of Serum and Vaccines (Prague, Czech Republic). Rimantadine tablets (commercial name Flumadin) were from Forest (Canada).

Apparatus

The dissolution paddle apparatus used was from Farmatest (Germany). Electrophoretic measurements were performed with a SpectraPhoresis 2000 (Thermo Bioanalysis Corporation, CA, USA) using uncoated fused-silica capillary tubing, inner diameter 75 μm (Avery Dennison, MA, USA), total capillary length 44 cm (36.5 cm to detector). The applied voltage was 30 kV, the temperature 25 °C. A PHM 64 (Radiometer, Copenhagen, Denmark) with saturated calomel electrode and glass electrode were used to measure pH. A Hewlett-Packard (Germany) HP08453 UV – visible spectrophotometer with a 1-cm path-length quartz cell was used for absorbance measurements.

CZE Analysis

Before use the capillary was washed for 5 min with 1 M NaOH at 60 °C, for 5 min with 0.1 M NaOH at 60 °C, and then for 10 min with water at 30 °C. Finally, the capillary was rinsed for 10 min with background electrolyte at 25 °C. Before each measurement, the capillary was washed with the working electrolyte.

Buffer solutions were filtered through glass S4 filters (Cavalier, Czech Republic); before use they were degassed in an ultrasonic bath (Branson, Shelton Conn, USA).

Content Uniformity

For determination of rimantadine five tablets were weighed and pulverized. A specific amount of the powder was weighed and mixed with water and the mixture was placed in ultrasonic bath for at least 15 min. The solution was then filtered and diluted to afford a final solution containing 30–50 ppm rimantadine.

Dissolution Testing

The dissolution of nine series of three tablets was followed for 30 min, using distilled water at 37 ± 0.5 °C as dissolution medium. The total volume of each vessel was 900 mL and the stirring speed was 60 rev min^{-1}. The method used was in accordance with USP and JP requirements [11].

Initially the supernatant from the dissolution samples was injected directly into the capillary CZE apparatus, but this resulted in high standard deviation and data dispersion. Subsequently, samples for CZE analysis were passed through 0.45-μm filters to remove the particles present after dissolution.

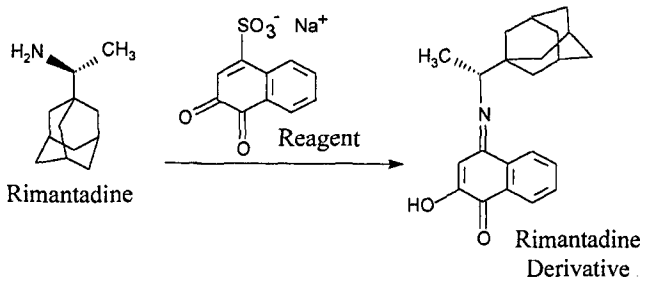

Figure 1
The derivatization of rimantadine with 1,2-naphthoquinone-4-sulfonic acid.

Results and Discussion

Derivatization

Rimantadine has only one amino group as a functional group. Derivatization for GC analysis is very complicated – it is necessary to extract the compound and work in non-aqueous media [6–8]. We have tried to avoid the use of organic solvents. Several derivatization reactions were studied. The reaction between rimantadine and 1,2-naphthoquinone-4-sulfonic acid (Figure 1) in alkali at high temperature furnishes a neutral orange derivative which precipitates [12]. Addition of various surfactants and cyclodextrins was studied to find conditions preventing precipitation of the derivative. The neutral surfactant Brij 35 was the most suitable; it keeps the derivative in solution thus enabling spectrophotometric determination in the UV/Vis range. The derivatization reaction was studied by spectrophotometry to optimize the reaction conditions before trying CZE determination of the rimantadine derivative.

The dependence of the efficiency on pH, buffer (concentration and type of buffer), concentrations of surfactant and 1,2-naphthoquinone-4-sulfonic acid reagent, reaction temperature, and reaction time was studied. It was found that no derivative was produced in acid media. Several buffers such as TRIS and tetraborate were tested in alkaline media; tetraborate resulted in the highest sensitivity. The absorption spectrum of the rimantadine derivative in tetraborate buffer has a maximum at 460 nm (Figure 2). To stabilize the solution of the derivatization product, several surfactant concentrations in the range 0.8 to 4 % were tested. For Brij 35 concentrations of 1.5 % (*w/v*) and above the absorbance was stable and constant for more than 50 min if 30 ppm of rimantadine was used. For rimantadine concentrations of 50–100 ppm the precipitate appeared after 10 min even when 1.5 % Brij 35 was used and a higher concentration of surfactant was therefore used to prevent precipitation. The optimum concentration was eventually established as 2.5 %.

Figure 2 shows how the spectrum of 1,2-naphthoquinone-4-sulfonic acid (reagent) changes as Brij 35 or

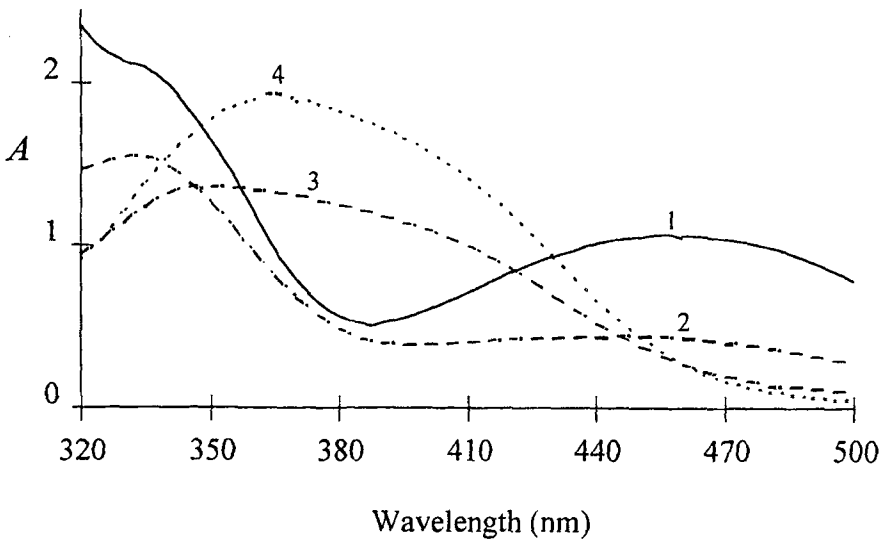

Figure 2
Absorption spectra (visible range) relevant to the derivatization of rimantadine: 1, 1.5 % Brij 35 + 17.5 mM tetraborate + 0.61 mM reagent + 50 ppm rimantadine; 2, 17.5 mM tetraborate + 0.61 mM reagent; 3, 1.5 % Brij 35 + 0.61 mM reagent; 4, 7.52 mM reagent.

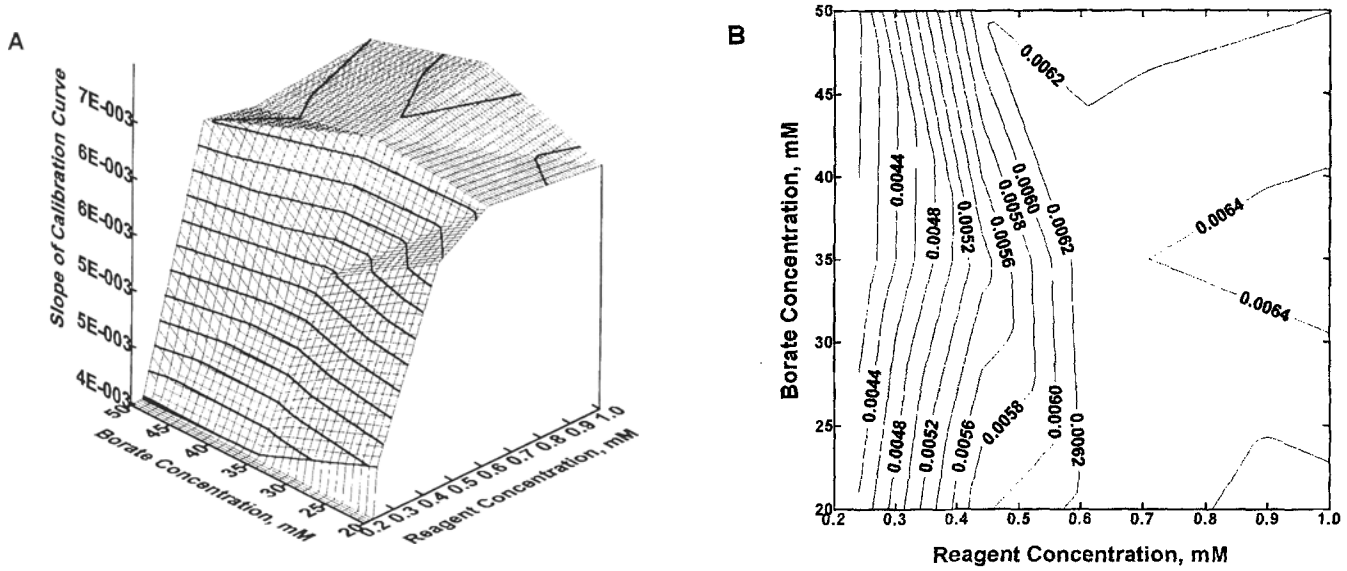

Figure 3
Dependence of the slope of the calibration curve on the concentrations of tetraborate and 1,2-naphthoquinone-4-sulfonic acid: A, response surface; B, corresponding contours of the response surface.

tetraborate are added. When tetraborate and Brij 35 were mixed together with the reagent, however, the spectrum obtained was identical with that obtained for the tetraborate – reagent mixture. Optimum tetraborate and reagent concentrations were also investigated. As is illustrated in Figure 3, the optimum concentrations of tetraborate and reagent are in the plateau region. Tetraborate and reagent concentrations of 40 mM and 0.7 mM, respectively, were chosen. The optimum pH of 9.2 was established for use of tetraborate.

The order of mixing of the compounds was also studied. The best results were obtained when the compounds were mixed in the order Brij 35, tetraborate, reagent, water, and rimantadine. The reaction process was promoted by elevated temperatures – it was finally performed in a boiling water bath to reduce the reaction time to 8 min.

As is illustrated in Figure 2, the rimantadine derivative alone has an absorption maximum at 460 nm. Brij 35 and excess 1,2-naphthoquinone-4-sulfonic acid did not interfere. The direct spectrophotometric analytical pro-

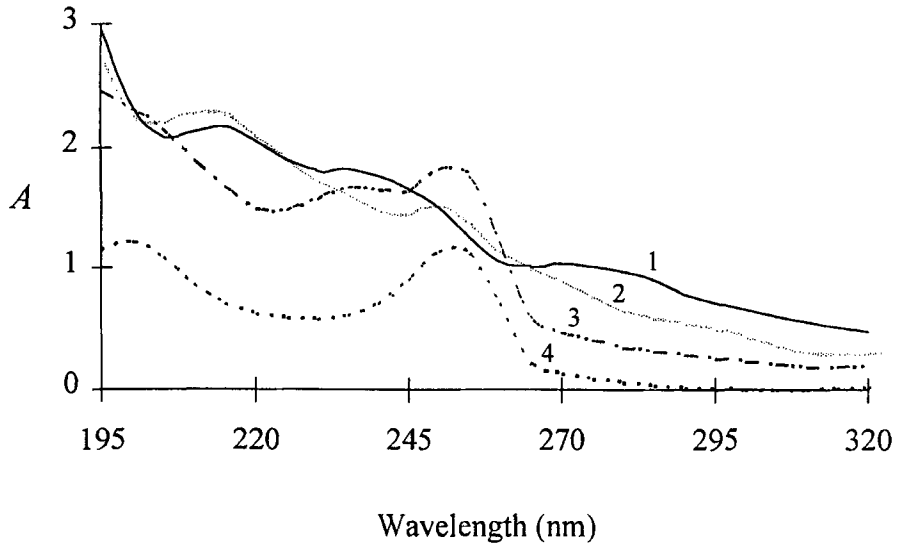

Figure 4
Absorption spectra (UV range) relevant to the derivatization of rimantadine: 1, 0.37 % Brij 35 + 4.37 mM tetraborate + 0.15 mM reagent + 12.5 ppm rimantadine; 2, 0.37 % Brij 35 + 4.37 mM tetraborate + 0.15 mM reagent; 3, 0.37 % Brij 35 + 0.15 mM reagent; 4, 0.68 mM reagent.

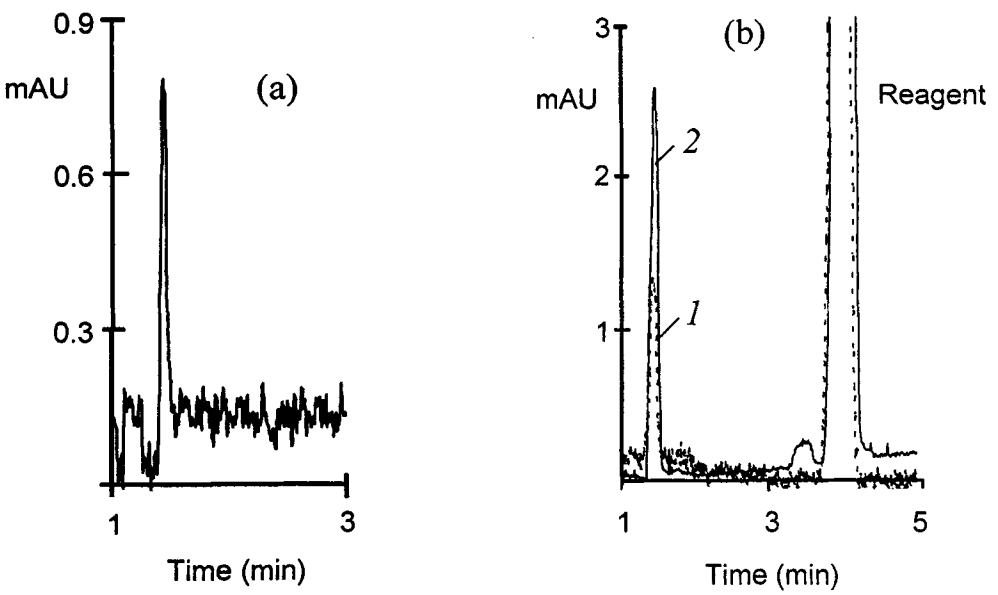

Figure 5
Electropherograms obtained after derivatization of rimantadine: (a) detection of 100 ppm rimantadine in the visible region at 460 nm; (b) detection in the UV region at 280 nm; 1, blank (see text); 2, 5 ppm rimantadine. Background electrolyte, 40 mM tetraborate, pH 9.2; injection, 25 s hydrodynamic; running voltage, 30 kV; temperature, 25 °C.

cedure is simple and rapid (see below) although in the UV range the analysis is difficult because the spectra of the blank (Brij 35 + tetraborate + reagent) and the rimantadine derivative (50 ppm) are quite similar (Figure 4), even if the sensitivity in UV range is higher.

Optimum Conditions

It is recommended the reaction is performed in 2.5 % (*w/v*) Brij 35, 40 mM tetraborate buffer, pH 9.2, 0.7 mM 1,2-naphthoquinone-4-sulfonic acid and heating the reaction mixture for 8 min on a boiling water bath. The reaction mixture (100 mL) is prepared dissolving surfactant (5.0 g), sodium tetraborate (3.051 g) and reagent (36.4 mg), each compound being dissolved before addition of the next. The reaction mixture (1.5 mL) is mixed with rimantadine solution (1.5 mL) and placed on a boiling water bath for 8 min. After cooling to room temperature in cold water after 10 min the absorbance of the derivative is measured at 460 nm against the blank.

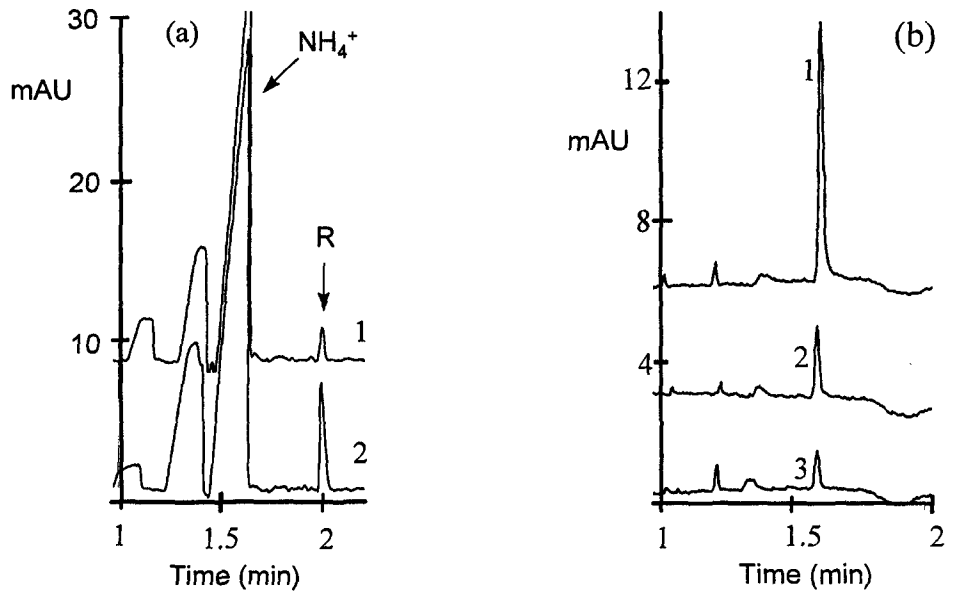

Figure 6

Electropherograms obtained from determination of rimantadine using indirect detection: (a) electrokinetic injection (10 s, 10 kV); (b) hydrodynamic injection (10 s). Background electrolyte, 5 mM 4-methylbenzylamine in 1:4 methanol-water; temperature, 25 °C; running voltage, 30 kV; detection at 210 nm. For the other conditions see text.

The blank is to be prepared by the same procedure using thrice-distilled water instead of rimantadine solution.

The reaction mixture should be prepared daily. Rimantadine is stable in aqueous solution for a few days only [9], so analysis should be performed on the same day as the samples are obtained; otherwise storage under refrigeration for no longer than 2 days is recommended.

CZE *Determination of Rimantadine after Derivatization*

When CZE analysis of rimantadine as its derivative with 1,2-naphthoquinone-4-sulfonic acid was tried using the optimum conditions found above for spectrophotometric analysis, problems were encountered. When analysis of the reaction mixture was performed in the visible range (460 nm) a small peak only was obtained for 100 ppm rimantadine (Figure 5a). The reason is the very short absorbance path-length inside the capillary (75 μm). Detection in the UV range (280 nm) was therefore studied but surfactant and derivative peaks comigrated (Figure 5b). Reducing the Brij 35 concentration to 1.5 % reduced the interference but necessitated rapid manipulation of the samples to avoid precipitation before analysis; another possibility was to reduce the working concentration range of rimantadine to 10–50 ppm. If this is done there is no problem applying the method to dissolution testing but care must be taken to keep the sample concentration inside this concentration range.

Indirect Detection

Rimantadine is almost transparent even in the far UV – it absorbs only slightly at 195 nm. The possibility of direct detection was studied using tetraborate and boric acid as background electrolyte (pH 9.2 and 5), but it was found that detection was possible only for rimantadine concentrations greater than 23 mM. Under these conditions rimantadine is present as the rimantadinium cation.

Indirect detection was studied using several absorbing background electrolytes. Buffer systems such as imidazole, pyridine, ephedrine, and 4-methylbenzylamine were tested. A solution of 4-methylbenzylamine in 1:4 methanol-water was found to be the most sensitive. The spectrum of this solution does not change in the pH range 3 to 10 and has two maxima (at 195 and 210 nm). That at 210 nm was chosen for indirect determination of rimantadine.

The effects of background electrolyte concentration, pH and, moreover, of the type and time of injection were studied. It was found that hydrodynamic and electrokinetic injection modes resulted in different electropherograms (Figure 6) and that the optimum conditions were different for each mode of injection – a background electrolyte concentration of 5 mM and of pH 9.0 was used with hydrodynamic injection (10 s) whereas with electrokinetic injection (10 s, 10 kV) the best results (high sensitivity and narrower peak) were obtained by use of 5 mM background electrolyte at pH 5.0. As is demonstrated in Figure 6, two or three unknown peaks were observed after electrokinetic injection. One, identified as ammonium, probably originated from the syn-

thesis of rimantadine. The other peaks are traces of unidentified cationic compounds present in the medicament. The detection limit for rimantadine was estimated as 0.1 ppm, but the reproducibility of the peak area was rather low (*RSD* up to 10 %); the results obtained from this injection mode depend dramatically on the composition of the sample.

Hydrodynamic injection results in simpler electropherograms and detection limits of approximately 2 ppm (Table I). Because low detection limits are not required for the determination of rimantadine in pharmaceutical preparations, hydrodynamic injection was preferred for this type of analysis. Electrokinetic injection, however, is a good alternative if low detection limits are needed.

Optimum Conditions

A solution of 4-methylbenzylamine (5 mM) in 1:4 methanol-water, pH 9.0, is used as background electrolyte. Hydrodynamic injection is performed for 10 s; the running voltage is 30 kV, the temperature 25 °C, and detection is performed at 210 nm. The background electrolyte is prepared by dissolving 4-methylbenzylamine (130 µL) in methanol (20 mL) and diluting to 100 mL with thrice-distilled water. The pH is adjusted to 9.0 by addition of a small amount of 1 M HCl and the solution is then filtered and degassed. The solution should preferably be prepared daily, but can be stored in the refrigerator if used within 3 days.

Separation of Amantadine and Rimantadine

The separation of rimantadine and amantadine was also studied by use of CZE with indirect detection. Separation was possible at pH 5.3 after hydrodynamic injection (Figure 7). Better sensitivity was obtained for amantadine than for rimantadine. Amantadine is present as a cation which has greater mobility than rimantadine. Electrokinetic injection was also evaluated but other peaks from the medicaments were found to interfere.

Purity Control and Dissolution Test

The two methods were compared for determination of the purity of Flumadin tablets containing rimantadine;

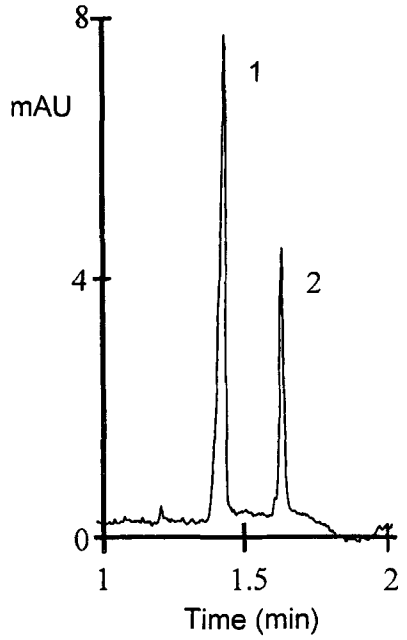

Figure 7
Separation of rimantadine and amantadine: 1, amantadine 0.7 ppm; 2, rimantadine, 15 ppm. Background electrolyte, 5 mM 4-methylbenzylamine in 1:4 methanol-water, pH 5.3; injection, hydrodynamic, 10 s; running voltage, 30 kV; temperature, 25 °C; detection at 210 nm.

Table II. Results from determination of rimantadine in Flumadin tablets by CZE with indirect detection or by spectrophotometric method after derivatization.

Method	Rimantadine content (mg tablet^{-1})[a]
CZE with indirect detection (peak area)	100.9 ± 1.0
Spectrophotometry	101.7 ± 0.5
Amount declared	100 ± 3.0

[a]Content ± standard deviation (n = 5)

Table I. Comparison of calibration curve parameters obtained by CZE with indirect detection or with spectrophotometry (after derivatization of rimantadine).

Method	Signal	R^2	Slope	Intercept	*LOD*[a] (ppm)	Linearity range[b] (ppm)	*RSD*[c] (%)
CZE	Peak height	0.9898	268.17	234.59	2.3	2.5–70	5.6
CZE	Peak area	0.9991	492.89	-223.3	1.6	1.5–100	1.4
Spectrophotometry	Absorbance	0.9835	6.74E-3	0.5416	2.0	2.0–120	0.8

[a]Limit of detection determined for signal-to-noise ratio (*S/N*) = 3 for CZE.
[b]Regression coefficient determined on the basis of 10 concentration levels. 65 and 75 points for CZE and spectrophotometry, respectively.
[c]Relative standard deviation determined for an analyte concentration of 50 ppm ($n = 4$) $RSD = (SD/50) \times 100$.

Table III. Results obtained from dissolution testing by use of CZE with indirect detection.[a]

Dissolution time (min)	Amount of rimantadine dissolved (mg L^{-1})[b]									Mean (%)
	A	B	C	D	E	F	G	H	I	
3	82.7 ± 2.6	78.7 ± 3.4	83.2 ± 3.8	81.3 ± 1.1	77.9 ± 4.7	81.7 ± 0.7	79.2 ± 2.9	87.4 ± 4.3	81.3 ± 1.3	81.49
4.5	84.7 ± 1.2	83.7 ± 2.5	85.7 ± 2.9	88.1 ± 3.4	80.6 ± 3.6	83.4 ± 2.3	84.4 ± 1.0	89.6 ± 4.5	84.6 ± 0.8	84.98
6	88.0 ± 3.1	85.4 ± 2.8	88.1 ± 1.1	89.0 ± 1.2	86.2 ± 3.2	88.1 ± 2.9	87.1 ± 3.5	90.1 ± 2.3	87.8 ± 3.7	87.76
9	89.6 ± 2.3	88.0 ± 1.9	89.3 ± 2.3	90.2 ± 2.6	89.5 ± 1.5	91.4 ± 3.4	89.7 ± 1.9	90.8 ± 2.8	90.9 ± 1.5	89.93
15	90.8 ± 0.7	89.9 ± 1.9	90.2 ± 1.8	90.9 ± 2.3	91.8 ± 2.8	93.2 ± 1.2	90.7 ± 2.4	91.7 ± 1.7	92.1 ± 2.6	91.26
30	96.9 ± 1.4	97.8 ± 2.0	96.1 ± 0.9	95.5 ± 1.0	98.5 ± 2.3	95.8 ± 2.7	96.7 ± 1.2	97.4 ± 2.2	96.8 ± 1.9	96.8

[a]These results were checked simultaneously by means of the spectrophotometric method described herein and agreement found to be acceptable.
[b]Amount dissolved ± standard deviation ($n = 3$).

CZE with indirect detection or with spectrophotometric detection after derivatization were both found to be suitable. The parameters of calibration curves constructed using CZE peak height and peak area were compared with those obtained by spectrophotometric analysis after derivatization (Table I); as expected, because of peak-shape distortion at higher concentrations, the use of peak area gave better results than peak height.

When rimantadine was determined by the spectrophotometric method the results were similar to those obtained by CZE but the linear range was greater. Detection limits for all three methods were approximately 2 ppm.

The results obtained for the rimantadine content of Flumadin tablets are summarized in Table II. The values are in a good agreement with the amount declared and acceptable standard deviations were obtained for both methods.

The dissolution testing of Flumadin tablets containing rimantadine was followed for 30 min by CZE with indirect detection. Table III lists the results obtained for the nine series of data. From these results it can be seen that after 3 min 70 % (78 mg L^{-1}) of rimantadine has dissolved. The standard deviation of the determination was < 5 %. It should be noted that the precision of the analytical method is much better than this; the rather high standard deviation arises from the dissolution procedure used for the tablets.

Conclusions

Better results are obtained for the determination of rimantadine by CZE when indirect detection with 4-methylbenzylamine is used rather than determination of rimantadine after derivatization. Indirect detection after hydrodynamic injection is more reproducible than after electrokinetic injection and the results are simpler. Detection limits of 0.1 ppm can be achieved by use of electrokinetic injection. Separation of rimantadine from amantadine is also possible after hydrodynamic injection. Spectrophotometric determination of rimantadine after derivatization with 1,2-naphthoquinone-4-sulfonic acid is an acceptable alternative method.

Acknowledgments

AR would like to thank the National Autonomous University of Mexico for its invaluable support.

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Received: Sep 1, 1997
Revised manuscript received: Nov 17, 1997
Accepted: Dec 2, 1997