DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 2, pp. 125–134, 2004

RESEARCH PAPER

# Validation of a Capillary Zone Electrophoresis Method for Determination of Rimantadine Hydrochloride in Rimantadin100 Tablets and the Method Application to Dissolution Test Monitoring

**J. Pazourek,* A. L. Revilla, D. Gajdošová, and J. Havel**

Department of Analytical Chemistry, Faculty of Science,
Masaryk University, Brno, Czech Republic

## ABSTRACT

A capillary zone electrophoretic method with indirect UV-detection for determination of rimantadine, an antiviral drug against influenza A, in tablets was validated. Instrumental precision, the method precision, accuracy, calibration curve linearity, selectivity, robustness, and time stability of the sample and the standard were tested. The method was also applied to monitor dissolution tests of the tablets. The possibility of addition of an internal standard for improvement of the method precision was discussed.

*Key Words:* Capillary zone electrophoresis; Adamantane; Rimantadine; Validation; Internal standard; Tablets; Antivirotics.

## INTRODUCTION

Potent antiviral activity has stimulated interest in the separation and determination of adamantane derivatives in the pharmacological field. One of the main categories of antiherpes agents presently used in chemotherapy is rimantadine [α-methyltricyclo (3,3,1,13,7)decane-1-methanamine] that effectively suppresses virus adsorption[1,2] and therefore was also utilized for treatment of the influenza A virus infection and prophylaxis.[3,4] Rimantadine is used to prevent or treat certain influenza infections (type A). The tablets Rimantadin100™ (Pliva-Lachema, Brno, Czech Republic) are undergoing approval tests in the Czech Republic.

The number of applications of capillary electrophoresis (CE) in the field of pharmaceuticals is rapidly growing since CE shows excellent selectivity and efficiency. Validation is the necessary step following analytical method development. Several authors have dealt with validation of analytical separation methods

*Correspondence: J. Pazourek, Department of Analytical Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, Brno, CZ-611 37, Czech Republic; E-mail: pazourek@chemi.muni.cz.



DOI: 10.1081/DDC-120028707
0363-9045 (Print); 1520-5762 (Online)

to various extents.[5–14] There are guides for method validation[15–20] also in the pharmaceutical literature, but being often derived from HPLC, their generality is often not sufficient for a particular application of interest. A procedure of validation was suggested specially for biochemical application.[21]

Because until now there is no commonly accepted guide for validation protocol in capillary electrophoresis, the goal of this paper is to validate the method suggested previously for determination of rimantadine hydrochloride[22,23] in Rimantadin100 tablets by capillary zone electrophoresis and to show the experimental steps of the validation protocol in detail. We also applied the method to monitoring the tablet dissolution process, which is a standard test for tablets under approval tests. Possibility of an internal standard addition to improve precision of the method was also discussed.

## EXPERIMENTAL

### Chemicals

Rimantadine hydrochloride (RIM) was purchased from Sigma Aldrich (Prague, Czech Republic) and 4-methylbenzylamine from Fluka (Buchs, Switzerland). Ethanol, ammonium nitrite, tetraethyl ammonium iodide, tetrabutyl ammonium hydroxide, sodium hydroxide, and hydrochloric acid were of analytical grade supplied by Lachema a.s. (Brno, Czech Republic). Bidistilled water used was produced in a quartz apparatus from Heracus (Hanau, Germany).

The matrix of the tablet (placebo) in powder form (Pliva-Lachema, Czech Republic) was used in some experiments as a blank.

### Apparatus

Spectraphoresis 2000 (TSP, San Jose, CA) was equipped with an unmodified fused silica capillary of Composite Metal Services Ltd. (The Chase, Hallow, Worcester, UK), inner diameter of 75 μm, total length 43 cm, effective length 36.5 cm. Injection was done by applying a vacuum of 10.4 kPa (1.5 psi) at the outlet capillary end. The apparatus was connected to a PC, and data acquisition and electropherograms evaluation was done with a PC1000 software of TSP (San Jose, CA). All experiments were done at 25°C. The pH was measured with a combined glass electrode at a pH meter OP-204 (Radelkis, Hungary). The dissolution apparatus was Erweka DT6 (Erweka GmbH, Düsseldorf, Germany) containing six 1000-milliliter vessels with paddles.

### Description of the Determination Procedure (Operation Protocol)

#### Determination of Rimantadine in Tablets—Principle

A tablet homogenate was dissolved in water to the final concentration of RIM at approximatively 100 mg/L. The content of RIM was determined by the method of capillary zone electrophoresis with indirect UV-detection. From the electropherograms peak area was read out and the mass of RIM in the tablet was calculated by the means of calibration curve of external standards (pure standard solutions without matrix).

#### Sample and Blank Preparation

The dissolving solution was distilled water. Twenty tablets were weighed, pulverized, and homogenized. Approximately 30 mg of the powder was quantitatively flushed out in a 100-mL calibrated flask, water was added, and the content was properly mixed and immersed into an ultrasonic bath for 30–60 seconds. Due to some insoluble compounds in the tablet matrix, before analysis the solution was filtered through a green disposable disc filter (Sleicher and Schüll #463513, green, pore diameter of 0.45 μm) into a electrophoretic vial (0.7 or 1.8 mL). The filtration can be avoided when working with solutions of standards.

The powder of matrix (blank) was homogenized in water, and the total matrix concentration was 1.002 g/L.

#### Background Electrolyte Preparation

Forty milliliters of ethanol were loaded into a 200-milliliter volumetric flask, and 130 microliters of 4-methylbenzylamine was added (to final concentration 5 mM). The flask was then carefully filled with distilled water up to the mark while the flask content was continuously mixed in order to avoid formation of white precipitate of recrystallized 4-methylbenzylamine. The solution was sonicated for approximately 20 seconds to remove air bubbles and make the solution transparent. The pH was adjusted by several drops of 1 M hydrochloric acid to 9.0. The solutions were filtered through glass S4 filters (Kavalier, Sazava, Czech Republic) and then degassed in an ultrasonic bath.

#### Instrumental Set-Up

The experimental conditions were optimized previously[22] for Spectraphoresis 2000 apparatus: fused



MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

silica capillary (unmodified), internal diameter 75 μm, total length 43 cm (effective length 36.5 cm), separation voltage was +20 kV, background electrolyte (BGE) was 5 mM 4-methylbenzylamine in 20% ethanol, hydrodynamic injection for 5 s (at 1.5 psi), indirect detection at 210 nm, time of experiment was 3 minutes, capillary filling with electrolyte for 30 seconds, and temperature was 25°C.

### Directions to Carry Out Experiments

At the beginning of an experiment series (daily), the capillary was washed for 5 min with 1 M NaOH, 5 min with 0.1 M NaOH, and then 10 min with water. Finally, the capillary was rinsed for 10 min with the background electrolyte. Before each measurement, the capillary was washed with the working electrolyte. Before the end of the series of runs (daily), a 2-minute wash with 0.1 M NaOH and water, respectively, was done. To check a good capillary performance (baseline and retention time stability), a run with standard solution of rimantadine was performed. The migration time of analyte was always between 2.1 and 2.4 minutes.

### Calibration Procedure

Twenty mg of RIM standard was weighed and quantitatively flushed out into a 100-milliliter volumetric flask. The flask was filled up to the mark and mixed. From this stock solution the standard solutions were prepared into 10 mL-volumetric flasks by taking 2.50, 3.00, 5.00, 7.00, and 7.50 milliliters, respectively. All the flasks were filled up to the mark and mixed. This way the calibration solutions with concentrations 50, 60, 100, 140, and 150 mg/L, respectively, were prepared. These solutions were analyzed under identical conditions as the samples (three runs with each solution). The calibration graph obtained from peak areas was evaluated by the linear regression. Calibration should be performed as often as necessary, at least after capillary replacement or before a long series of measurements.

### Calculations of the Active Compound Mass

The mass of the active compound in an average tablet can be calculated according to the following formula:

$$x = (A - a) \times M \times 0.1/(m \times b) \quad (1)$$

where A=RIM peak average area (μAU.s), a=calibration curve intercept (μAU.s), m=weighted homogenized powder mass (mg), M=mass of an average tablet (mg), and b=calibration curve slope (μAU.s.L/mg).

## Dissolution Tests

A tablet was dropped in the dissolution vessel under constant paddle stirring at time zero. Samples were taken at given time periods (3, 6, 9, 15, 30, 60, and 120 minutes) 3.3 cm beyond the medium level by a 5-mL plastic syringe (volume of 2–3 mL). The dissolving medium was distilled water of volume of 900 mL. Temperature of the medium was kept at 37±0.5°C. Stirring speed was set at 50 rpm.

### Sampling

About 3 milliliters of the solution from the dissolving vessel (900 mL) was loaded into a plastic syringe and was immediately filtered through a green disposable disc filter (Sleicher and Schüll #463513, green, pore diameter of 0.45 μm) into an electrophoretic vial (0.7 or 1.8 ml). The vials were inserted into the carousel of the Spectraphoresis 2000 apparatus. Always three repeated runs were performed from each vial.

### Calculation of Analyte Content Released from a Tablet

a) Percentage content of analyte released from a tablet relative to the theoretical mass of analyte (100.0 mg) was calculated according to the equation

$$\%x = 100 \times (A - a) \times V \times k/(b \times T) \quad (2)$$

where A=measured peak area of analyte (μAU.s), a=intercept of calibration curve (μAU.s), V=dissolution medium volume (L), k=ratio of theoretical content of analyte to the tablet mass (k=3.00 for uncoated tablet, k=3.06 for coated ones), b=slope of calibration curve (μAU.s.L/mg), T=mass of the tablet (mg).

b) Percentage content of analyte related to maximum released from a tablet was calculated according to the equation

$$\%x = A/Amax \times 100 \quad (3)$$



Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

where A=analyte peak area from a current sampling, and Amax=analyte peak area measured from the final sampling.

## RESULTS

### Validation Protocol

The method validation consists of the following steps, which should document the ability of the system to determine the analyte within declared (or acceptable) accuracy and precision. We tested the following parameters of the determination method: instrumental precision, method precision, method accuracy, linearity of calibration curve, selectivity, robustness, and the stability of standards and samples.

#### Test of Repeatability (Instrumental Precision)

One of the standard solutions (concentration of 101 mg/L) was repeatedly injected (from the same vial) and the migration time and peak area were statistically evaluated as shown in Table 1 (n=10). Both the migration time and peak area relative standard deviation were less than 1%.

#### Test of the Method Precision

Three tablets were pulverized and homogenized. Approximately 30 mg of the powder was ten times weighed, dissolved in a 100-mL volumetric flask, and the solution three times analyzed. An average migration time and an average ratio peak area/mass of the weighed sample (A/m) was statistically evaluated as shown in Table 2. Relative standard deviation (RSD) for migration time was <1%, and RSD of the ratio of peak area to weighed mass was <2%. These values are higher comparing to HPLC, but typical for CE methods.

***Table 1.*** Test of precision (instrumental precision).

| Experiment number | Migration time (min) | Peak area [μAU.s] |
|---|---|---|
| 1 | 2.302 | 32,767 |
| 2 | 2.294 | 33,019 |
| 3 | 2.293 | 32,974 |
| 4 | 2.288 | 32,969 |
| 5 | 2.281 | 33,303 |
| 6 | 2.280 | 33,062 |
| 7 | 2.278 | 33,494 |
| 8 | 2.275 | 33,372 |
| 9 | 2.274 | 33,235 |
| 10 | 2.273 | 33,043 |
| Number of measurements (n) | 10 | 10 |
| Average (∅) | 2.284 | 33,123.7 |
| Standard deviation $s=\sqrt{\frac{\sum(x-\varnothing)^2}{n-1}}$ | 0.010 | 221 |
| Relative standard deviation (RSD) %RSD=100*s/∅ | 0.43% | 0.67% |

***Table 2.*** Test of the method precision.

| Measurement # | Migration time (min) | Area/mass [μAU.s/mg] |
|---|---|---|
| 1 | 2.406 | 967.8 |
| 2 | 2.403 | 993.9 |
| 3 | 2.418 | 747.2* |
| 4 | 2.414 | 982.4 |
| 5 | 2.413 | 984.1 |
| 6 | 2.431 | 970.6 |
| 7 | 2.438 | 962.5 |
| 8 | 2.439 | 957.2 |
| 9 | 2.436 | 955.6 |
| 10 | 2.435 | 937.9 |
| Number of measurements (n) | 10 | 9 |
| Average (∅) | 2.423 | 968.0 |
| Standard deviation $s=\sqrt{\frac{\sum(x-\varnothing)^2}{n-1}}$ | 0.015 | 17.1 |
| Relative standard deviation $\%s_R=100*s/\varnothing$ | 0.6% | 1.8% |

The experiment with asterisk (*) was excluded according to the Grubss test, T(0.05,10)=2.228.

#### Test of the Method Accuracy

The method accuracy was done by the standard addition method. Into 10-milliliter volumetric flasks with 2.0 milliliters of the matrix solution, 2.50, 3.00, 5.00, 7.00, and 7.50 mL, respectively, of standard solution (c=218.6 mg/L) were added and the flasks were filled up to the mark. Always two parallel solutions were prepared. The peak areas were read out, a calibration curve was obtained by linear regression, and recovery (%R) was calculated according to the formula:

$$\%R = 100 \times Ci/C0 \quad (4)$$

where Ci=concentration calculated from the calibration curve and CO=concentration prepared. Results are summarized in Table 3. The results prove an important fact that the presence of matrix does not significantly affect the signal of RIM standards, therefore a calibration curve of external standards (standards of

***Table 3.*** Test of method accuracy.

| Sample number | C0 (mg/L) | Ci (mg/L) | % R |
|---|---|---|---|
| 1 | 54.65 | 52.951 | 96.89 |
| 2 | 54.65 | 52.725 | 96.48 |
| 3 | 65.58 | 66.784 | 101.84 |
| 4 | 65.58 | 66.939 | 102.07 |
| 5 | 109.30 | 111.017 | 101.57 |
| 6 | 109.30 | 111.460 | 101.98 |
| 7 | 153.02 | 147.541 | 96.42 |
| 8 | 153.02 | 152.270 | 99.51 |
| 9 | 163.95 | 163.732 | 99.87 |
| 10 | 163.95 | 167.575 | 102.21 |
| Number of measurement | n | | 10 |
| Average | ∅R | | 99.88 |
| Standard average | $s = \sqrt{\frac{\sum(x-100)^2}{n}}$ | | 2.325 |
| Relative standard deviation | $\%s_R = 100*s/\varnothing R$ | | 2.33% |
| Student coefficient | $t(\alpha=0.05,10)$ | | 2.2622 |
| Reliability interval | $IS = t.s/\sqrt{n}$ | | 1.664 |
| Requirement: ∅R lies into the interval | 100 ± IS | | fulfilled |

pure RIM) can be used for calibration of RIM in Rimantadin100 tablets.

## Test of Linearity of the Calibration Curve

Twenty mg of RIM standard was weighed and quantitatively flushed into a 100-milliliter volumetric flask. The flask was filled up to the mark and mixed. From this stock solution the standard solutions were prepared into 10-mL volumetric flasks by taking 2.50, 3.00, 5.00, 7.00, and 7.50 milliliters, respectively (five calibration point, three replicates). All the flasks were filled up to the mark and mixed. Solutions with concentration in the range 50–150% of the expected analyte concentration were prepared this way. These solutions were analyzed under identical conditions as the samples (three runs with each solution). The peak areas were used for construction of a linear regression curve and its parameters were statistically tested for linearity as shown in Table 4. To visually illustrate the linearity of the calibration curve, residuals calculated

***Table 4.*** Test of linearity.

| Sample (m=5) | Concentration (mg/L) | Area [μAU.s] (k=3) | | |
|---|---|---|---|---|
| 1 | 54.65 | 21,738 | 22,051 | 22,196 |
| 2 | 65.58 | 26,978 | 26,763 | 26,841 |
| 3 | 109.30 | 45,532 | 46,002 | 45,729 |
| 4 | 153.02 | 63,421 | 63,134 | 63,481 |
| 5 | 170.51 | 71,175 | 73,392 | 73,159 |
| Intercept | | b0 | −1,431.1 | |
| Slope | | b1 | 429.767 | |
| Correlation coefficient | | r | 0.9993 | |
| Standard deviation | | $s_e$ | 783.0 | |
| 1% of average y | | AY | 461.1 | |
| Reliability interval (0.05, 15) | | $c_1$ | 436.7 | |
| F=MSlof/MSpc | | | 4.76 | |
| F(0.01,3,10) | | | 6.55 | |
| Requirements no residuals $>2s_e$ | | | fulfilled | |
| Requirements $c_1<0.01$ of AY | | | fulfilled | |
| Linearity requirement F<F(0.01,3,10) | | | fulfilled | |

The data were evaluated by linear regression. AY is one hundredth of average area from all the 15 measurements.

***Table 5.*** Test of linearity.

| Concentration (mg/L) | Area residuals [μAU.s] | | |
|---|---|---|---|
| | k=1 | k=2 | k=3 |
| 54.65 | −317.5 | −4.5 | 140.8 |
| 65.58 | 224.6 | 10.1 | 87.7 |
| 109.30 | −10.8 | 459.9 | 186.8 |
| 153.02 | −911.1 | −1,197.8 | −851.4 |
| 170.51 | −672.4 | 1,544.1 | 1,311.3 |

Residuals of the calibration curve from Table 4.

as differences between each point and the regression line were calculated. The results are in Table 5. From signs and magnitudes of the residuals we could conclude that there was no systematic deviation of the calibration curve from linearity.

## Test of the Method Selectivity

Because of variability of applicable methods (HPLC, photometry, CZE, ITP, etc.), there is no universal procedure to demonstrate selectivity. Selectivity ensures that the signal measured is not influenced by matrix substances. For our case, we decided to first visually demonstrate the selectivity of the method as illustrated in Fig. 1: the lower electropherogram was obtained from a run with matrix solution (c=200 mg/L), the upper one from a run of the sample (RIM concentration of 106 mg/L).

The selectivity (SEL%) was also quantified by three repeated injections of the sample with the known content of the analyte and by evaluation of a formula:

$$SEL(\%) = 100 - 100 \times \frac{|A_0 - A_T|}{A_0} \qquad (5)$$

where $Ao$=area calculated from the calibration curve equation for the known concentration and $A_T$=average peak area obtained experimentally. At concentration of 109.3 mg/L, $A_0$ was 45,542 μAU.s, and $A_T$ was 45,822 μAU.s, SEL % was calculated as 99.0. Requirement of SEL≥99% was fulfilled.

## Test of the Method Robustness (Ruggedness)

Since pH is an important variable affecting the analyte migration, we tested robustness of the determination method by examining applicable pH range. We found that a change in pH by ±0.5 did not shift the migration time out of the range of 2.1–2.4 minutes. The pH change could influence resolution if a contaminant with mobility similar to analyte (e.g., adamantanamine) was present, which was, however, not so in the case of Rimantadin100 tablets.

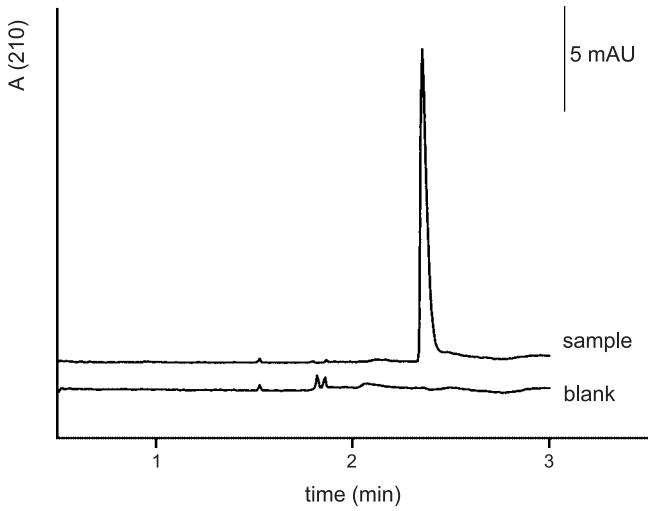


***Figure 1.*** Method selectivity. The lower electropherogram was obtained from a run with a blank solution (matrix), the upper one from a run of the sample (homogenized tablets with RIM concentration of 106 mg/L). Experimental conditions: fused silica capillary (unmodified) I.D. 75 μm, length 43 (36.5) cm, separation voltage was +20 kV, BGE was 5 mM 4-methylbenzylamine in 20% ethanol, hydrodynamic injection for 5 s (at 1.5 psi), indirect detection at 210 nm, and temperature was 25°C.

## Test of Sample and Standard Stability

The sample and standard stability is an important factor to be considered when a long series of analyses are performed, typically when the samples are queued in an autosampler or kept in a refrigerator before the analysis. The sample stability should guarantee that the concentration of analyte in a sample is kept

***Table 6.*** Sample and standard stability.

| | INS (4 h) % | INS (8 h) % |
|---|---|---|
| Standard, t=25°C | 2.4 | 2.9 |
| Standard, t=5°C | 0.0 | 2.2 |
| Tablet, filtrate=25°C | −0.5 | 1.8 |
| Tablet, filtrate t=5°C | −1.5 | 0.6 |

The values were calculated according to Eq. (6).

practically constant from the time of sampling to the time of analysis.

We quantified the stability as the following: a solution of standard of RIM was prepared (approximately from 10 mg). Also, a filtrate of a dissolved tablet was prepared (approximately from 30 mg), and both solutions were run and divided into two parts: one part was kept at room temperature (20–25°C) and the second part in a refrigerator (temperature of 4–6°C). These solutions were analyzed after a period of 4 hours. Prior to each run, fresh solution of standard and tablet, respectively, were prepared and analyzed. A time

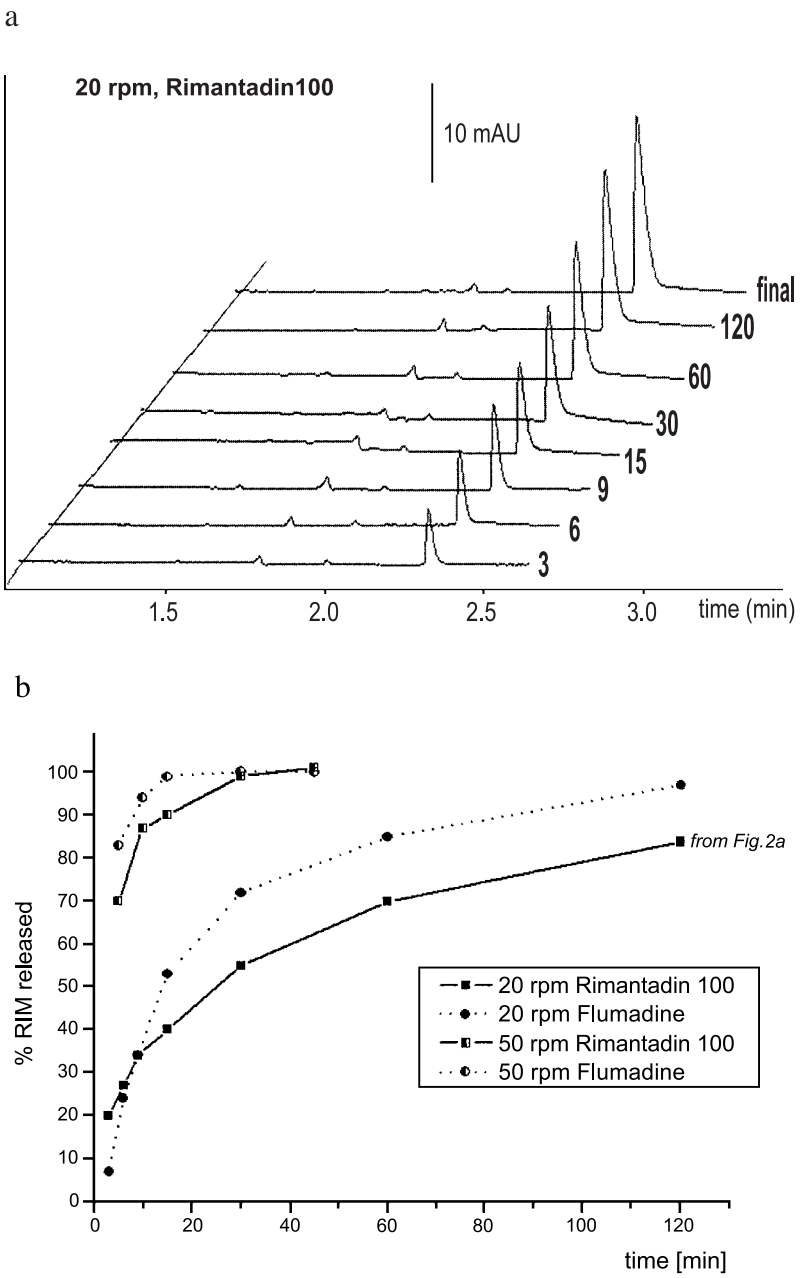


***Figure 2.*** Dissolution profiles of Rimantadin100 and Flumadine tablets. In Fig. 2a (20 rpm) the electropherograms are marked according to the increasing sampling time: 3, 6, 9, 15, 30, 60, 120, and final, respectively. In Fig. 2b there are graphs of % RIM released from tablets at dissolution tests. Calculations are made according to Eq. 3 (Amax was obtained from the final sampling). The half-closed symbols hold for 50 rpm, full closed symbols for 20 rpm.

instability of the solutions was calculated according to the formula:

$$INS_t(\%) = 100 \times \frac{A6_T - A6}{A6} \quad (6)$$

where A6=average of six measurements of peak areas of a fresh solution, $A6_T$=average of six measurements of a stored solution.

Deducing from both the absolute values and signs of instability (INS), we can conclude that the value of INS of tablet solution was not significantly different from that of standard solution (see Table 6). Both the aqueous solution of tablet and the standard are stable after 8 hours at room temperature (e.g., in an autosampler).

### Dissolution and Stability Test of Tablets of Rimantadin100

In tablets of Rimantadin100 that contain RIM, the validated procedure described above was applied. The only difference was that the samples were solutions taken from dissolution medium from a liter vessel, where the tablets were being dissolved (medium volume of 900 mL). Because the average content of RIM in a tablet was 100 mg, the expected concentration in the samples falls within the calibration range 50–150 mg/L.

The speed of 50 rpm is typically used in common dissolution tests because the released analyte is quickly distributed in the medium. At this speed, approximately 70% of RIM was released in 5 minutes and 100% in 30 minutes, respectively. Typically, the requirement for the approval tests is that 90% of mass of the analyte should be released within 30 minutes at 50 rpm, which was fulfilled [calculation from Eq. (2)].

To obtain the tablet dissolution profiles where a systematic increase in peak area is shown, we used 20 rpm and fractions were collected in times of 3, 6, 9, 15, 30, 60, and 120 minutes, respectively. To obtain a real maximum of analyte released in the medium, after the last sampling the solution was homogenized (by fast stirring) and a final sampling was performed. The results illustrating the dissolution process are in Fig. 2a: electropherograms are cascaded according to the time of sampling. The increasing peak area corresponds to the increasing amount of RIM released into the medium. In Fig. 2b there is a graph of the process expressed as mass percentage of the analyte released into the dissolving solution [calculation according to Eq. (3)] compared to RIM released under the same experimental conditions from an analogous drug Flumadine (Forest Laboratories). The comparison shows that the rate of dissolution is higher at Flumadine tablets. It can be explained by the fact that the tested Flumadine tablets are uncoated while Rimantadin100 tablets are coated ones.

## DISCUSSION

### The Possibility of the Use of an Internal Standard

A relatively low precision (reproducibility) of capillary electrophoresis methods compared to HPLC is well known. Therefore, we tested the three following compounds as internal standards (abbreviation and concentration are given in parenthesis), which could improve precision of the method: ammonium nitrite ($NH_4$, 12.5 mg/L), tetraethyl ammonium iodide (TEA, 15 mg/L), and tetrabutyl ammonium hydroxide (TBA, 12.5 mg/L). To pronounce an effect of the internal standards, the concentration of RIM was lowered to 12.5 mg/L. The three compounds were added to an aqueous solution of RIM standard and the mixture was repeatedly analyzed under experimental conditions identical to those of RIM determination. For all peaks with resolution higher than 1.5, retention times, areas, and peak heights were measured and their relative standard deviations were calculated.

The results are graphically shown in Figs. 3 and 4. From Fig. 4b it can be seen that points 8 and 9 are outliers with respect to RIM peak height or the ratio of RIM peak height to ammonium peak height. However, ratios of heights for the same points do not exhibit a significant change, which means that these internal standards corrected the peak height fluctuation.

The experiments showed that RSD of peak areas was comparable to RSD of peak heights and the best internal standard was TEA. Further, RSD of RIM peak area and RSD of ratio of RIM peak area to TEA peak area were 1.9% and 3.3%, respectively. Therefore, the most important conclusions is that the use of the

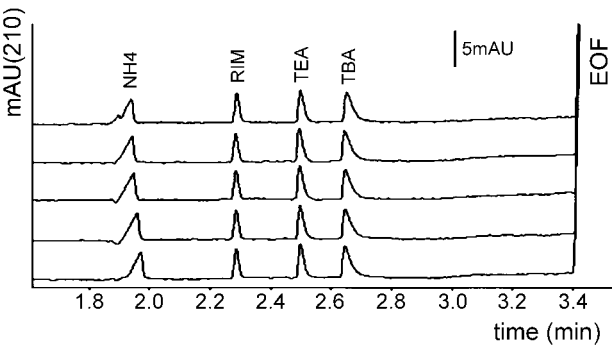


***Figure 3.*** Test of compounds as internal standards.

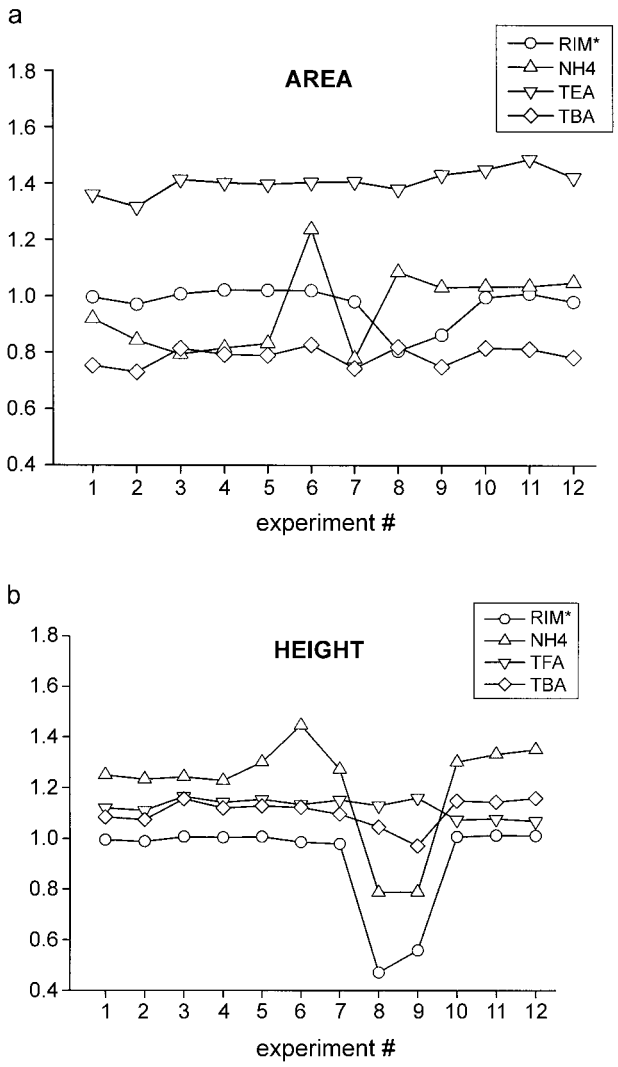


***Figure 4.*** Internal standard use. The y-axis is in relative units in order to graphically compare the courses of the graphs. Open circles (RIM*) denote peak area (Fig. 4a) and heights (Fig. 4b), respectively, of RIM relative to their first values. Other symbols denote always peak area and heights, respectively, of RIM relative to the particular internal standard.

tested internal standards did not improve the precision. The reason for the use of internal standard might be in the correction of outliers (sudden change in migration time has the same tendency for both the analyte and internal standard).

## CONCLUSIONS

The method for determination of rimantadine hydrochloride in tablets Rimantadin100 was validated and the validation protocol applicable to CE methods was presented. The analysis is fast (less than 4 minutes) and can be also applied to dissolution profile tests of Rimantadin100 tablets or used to determine RIM in analogous pharmaceuticals. The use of tested internal standards (ammonium nitrite, tetraethyl ammonium iodide, tetrabutyl ammonium hydroxide) did not bring improvement in determination precision.

## REFERENCES

1. Antoniadou-Vyza, E.; Avramidis, N.; Kourounakis, A.; Hadjipetrou, L. Anti-inflammatory properties of new adamantane derivatives. Design, synthesis, and biological evaluation. Arch. Pharm. Pharm. Med. Chem. **1998**, *331* (2), 72–78.
2. Lin, T.; Heider, H.; Schroeder, C. Different modes of inhibition by adamantane amine derivatives and natural polyamines of the functionally reconstituted influenza virus M2 proton channel protien. J. Gen. Virol. **1997**, *78*, 767–774.
3. Tsunoda, A.; Maasab, H.F.; Cochran, K.W.; Eveland, W.C. Antiviral activity of alpha-methyl-1-adamantanemethylamine hydrochloride. Antimicrob. Agents Chemother. **1965**, *5*, 553–560.
4. Hayden, F.G.; Minocha, A.; Spyker, D.A.; Hoffman, H.E. Comparative single-dose pharmacokinetics of amantadine hydrochloride and rimantadine hydrochloride in young and elderly adults. Antimicrob. Agents Chemother. **1985**, *28* (2), 216–221.
5. Altria, K.D.; Goodall, D.M.; Rogan, M.M. Quantitative applications and validation of the resolution of enantiomers by capillary electrophoresis. Electrophoresis **1994**, *15* (6), 824–827.
6. Sänger-van de Griend, C.E.; Gröningsson, K. Validation of a capillary electrophoresis method for the enantiomeric purity testing of ropivacaine, a new local anaesthetic compound. J. Pharm. Biomed. Anal. **1996**, *14* (3), 295–304.
7. Altria, K.D.; Walsh, A.R.; Smith, N.W. Validation of a capillary electrophoresis method for the enantiomeric purity testing of fluparoxan. J. Chromatogr. **1993**, *645* (1), 193–196.
8. Altria, K.D.; Chanter, Y.L. Validation of a capillary electrophoresis method for the determination of a quinolone antibiotic and its related impurities. J. Chromatogr. **1993**, *652* (2), 459–463.
9. Altria, K.D.; Filbey, S.D. Quantitative pharmaceutical analysis by capillary electrophoresis. J. Liq. Chromatogr. **1993**, *16* (11), 2281–2292.
10. Liu, L.; Osborne, L.M.; Nussbaum, M.A. Development and validation of a combined potency assay and enantiomeric purity method for a chiral

pharmaceutical compound using capillary electrophoresis. J. Chromatogr. **1996**, *745* (1–2), 45–52.
11. Swartz, M.E.; Mazzeno, J.R.; Grover, E.R.; Brown, P.R. Validation of enantiomeric separations by micellar eletrokinetic capillary chromatography using synthetic chiral surfactants. J. Chromatogr. **1996**, *735* (1–2), 303–310.
12. Thomas, B.R.; Ghodbane, S. Evaluation of a mixed micellar electrokinetic capillary electrophoresis method for validated pharmaceutical quality-control. J. Liq. Chromatogr. **1993**, *16* (9–10), 1983–2006.
13. Fillet, M.; Bechet, I.; Chiap, P.; Hubert, P.; Crommen, J. Enantiomeric purity determination of propranolol by cyclodextrin-modified capillary electrophoresis. J. Chromatogr. **1995**, *717* (1–2), 203–209.
14. Daali, Y.; Cherkaoui, S.; Christen, P.; Veuthey, J.-L. Experimental design for enatioselective separation of celiprolol by capillary electrophoresis using sulfated beta-cyclodextrin. Electrophoresis **1999**, *20* (17), 3424–3431.
15. ICH http:/www.ifpma.org, EURACHEM http://www.vtt.fi/ket/eurachem/valid.html, http://www.vam.org.uk/.
16. Huber, L. Equipment qualification in practice. LC GC Mag. Sep. Sci. **1998**, *16* (2), 148.
17. Validation of analytical methods: methodology. Pharmeuropa **1996**, *8* (1).
18. Vander Heyden, Y.; Nijbuis, A.; Smeyer-Verbeke, J.; Vandeginste, B.G.M.; Massart, D.L. Guidance for robustness/ruggedness tests in method validation. http://minf.vub.ac.be/~fabi/validation/robust/guideline.doc.
19. Clarke, G.S. The validation of analytical methods for drug substances and drug products in Uk Pharmaceutical Laboratories. J. Pharm. Biomed. Anal. **1994**, *12* (5), 643–652.
20. van Zoonen, P.; Hoogerbrugge, R.; Gort, S.M.; van de Wiel, H.; van't Klooster, H.A. Some practical examples of method validation in the analytical laboratory. TrAc **1999**, *18* (9–10), 584–593.
21. Hubert, Ph.; Chiap, P.; Crommen, J.; Boulanger, B.; Chapuzet, E.; Mercier, N.; Bervoas-Martin, S.; Chevalier, D.; Lagorce, P.; Lallier, M.; Laparra, M.C.; Laurentie, M.; Nivet, J.C. The SFSTP guide on the validation of chromatographic methods for drug bioanalysis: from the Washington conference to the laboratory. Anal. Chim. Acta **1999**, *39* (2), 135–148.
22. Revilla, A.L.; Hamáček, J.; Lubal, P.; Havel, J. Determination of rimantadine in pharmaceutical preparations by capillary zone electrophoresis with indirect detection or after derivatization. Chromatographia **1998**, *47* (7–8), 433–439.
23. Reichová, N.; Pazourek, J.; Polášková, P.; Havel, J. Electrophoretic behaviour of adamantane derivatives possessing antiviral activity and their determination by capillary zone electrophoresis with indirect detection. Electrophoresis **2002**, *23* (2), 259–262.



Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016