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The use of capillary electrophoresis to monitor the stability of a dual-action cephalosporin in solution ☆

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Abstract

Ro 23-9424 is a broad-spectrum antibacterial agent consisting of a cephalosporin and a quinolone moiety which are held together by an ester linkage. This compound has limited solubility in water but is more soluble at alkaline pH. Such high pH values, however, lead to stability problems due to the lability of the ester linkage, and result in the formation of the free quinolone and cephalosporin moieties. The balance between solubility and stability presents a challenge in formulation development for this compound. Thus, it is important to effectively monitor the stability of Ro 23-9424 after it has been reconstituted in different solvent systems. In this manner, a diluent which does not lead to degradation of the drug can be identified. A capillary electrophoresis method was developed and validated to monitor the stability of Ro 23-9424. The method was found to meet acceptable criteria for method precision, system precision, linearity and limits of detection and quantitation. The method was used to monitor the stability and measure the half-life of Ro 23-9424 in water and in an L-arginine–sodium benzoate–saline diluent designed to mimic the drug delivery system. This work shows that capillary electrophoresis can be used to compare the stability of a drug in different solutions as an aid in formulation development.

Keywords: Antibacterial agent; Capillary electrophoresis; Formulation development; Pharmaceuticals; Ro 23-9424; Stability monitoring

1. Introduction

Ro 23-9424 is a broad-spectrum antibacterial agent consisting of a cephalosporin (desacetylcefotaxime) and a quinolone (fleroxacin) moiety which are held together by an ester linkage. Ro 23-9424 exhibits the antibacterial spectrum and potency of both the cephalosporin and quinolone moieties [1–4]. Ro 23-9424 has limited solubility in water but is more soluble at alkaline pH. Such high pH values, however, lead to stability problems due to the lability of the ester linkage and result in the formation of the free quinolone and cephalosporin moieties. Fig. 1 shows the structure of Ro 23-9424 and its two degradation products, Ro 23-6240 (fleroxacin) and Ro 19-4885 (des-

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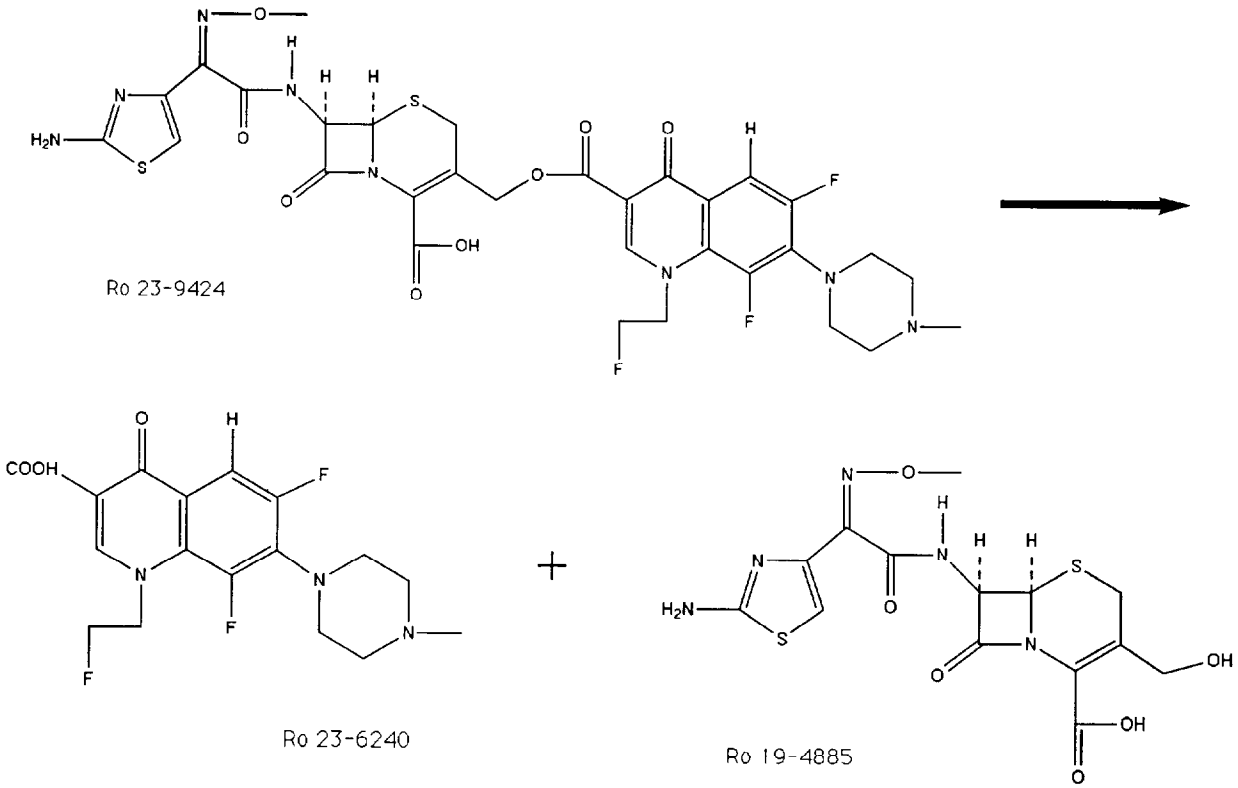


Fig. 1. Structure of Ro 23-9424 and its degradation products, Ro 23-6240 and Ro 19-4885.

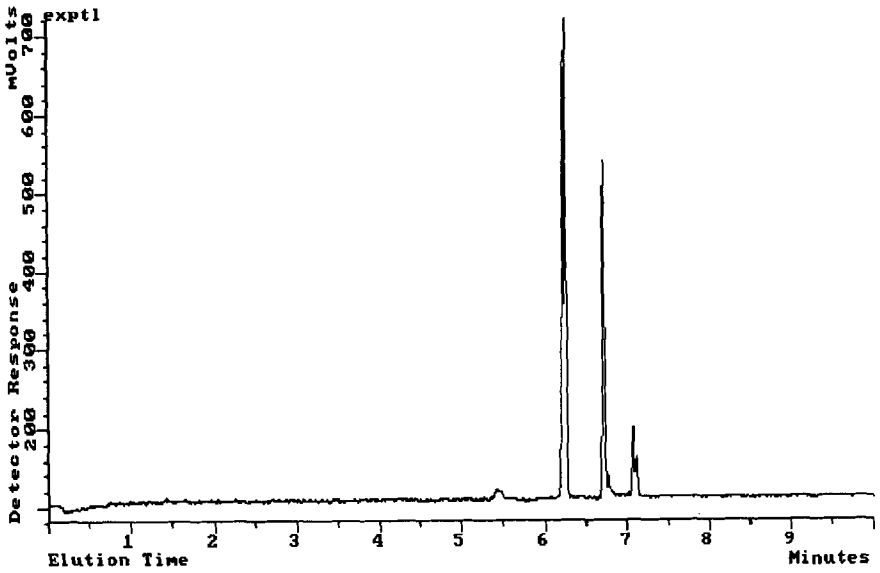
acetylcefotaxime). The balance between solubility and stability presents a challenge in formulation development for this compound. It is therefore important to effectively monitor the stability of Ro 23-9424 after it has been reconstituted in different solvent systems. In this manner, a diluent which does not lead to degradation of the drug can be identified.

This project represented an opportunity to evaluate the usefulness of capillary electrophoresis in a pharmaceutical application. Capillary electrophoresis, in the form of capillary zone electrophoresis and micellar electrokinetic chromatography, is a technique which has been used extensively to analyze pharmaceutical compounds [5–7] including quinolones [8] and cephalosporins [9–11]. Capillary electrophoresis has also been used to monitor the stability of compounds in solution [12] and to perform pharmacokinetic studies [13]. In the work presented here, a capillary electrophoresis method was developed to monitor the stability of Ro 23-9424 in solution. This method separates Ro 23-9424 from its two known degradation products, Ro 23-6240 and Ro 19-4885. The system precision, method precision, linearity and limits of detection and quantitation of the method were determined. The capillary electrophoresis method was used to monitor the stability of Ro 23-9424 in water and in an L-arginine–sodium benzoate–saline diluent. Information regarding the half-line of Ro 23-9424 in these two solutions was obtained. The applicability of capillary electrophoresis to monitor the stability of Ro 23-9424 as an aid in formulation development was demonstrated and is discussed.

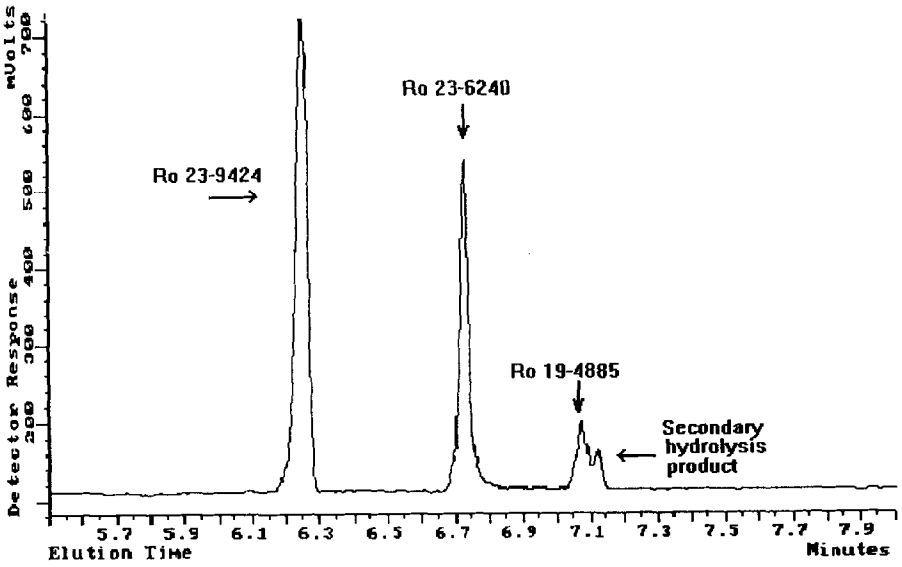
2. Experimental

2.1. Chemicals and reagents

The drug, Ro 23-9424, and its degradation products, Ro 23-6240 and Ro 19-4885, were manufactured by Hoffmann–La Roche Inc. (Nutley,



(a)



(b)

Fig. 2. (a) Full-scale electropherogram, and (b) expanded-scale electropherogram showing the separation of Ro 23-9424, Ro 23-6240, Ro 19-4885 and an unknown degradation product.

NJ). Boric acid (certified A.C.S. grade), sodium benzoate (U.S.P. grade), sodium borate (certified A.C.S. grade) and sodium chloride (certified A.C.S. grade) were obtained from Fisher Scientific Company (Fair Lawn, NJ). L-Arginine (USP reference standard) was obtained from the U.S. Pharmacopeial Convention, Inc. (Rockville, MD). Water was purified using a NANOpure system (Barnstead/Thermolyne Corporation, Dubuque, IA).

Table 1
Parameters for the separation of Ro 23-9424 and its impurities

Component	Migration time (min)	Tailing factor	Theoretical plates, N	Resolution factor, R_s
Ro 23-9424	6.25	0.76	164000	8.3
Ro 23-6240	6.73	0.99	256000	6.2
Ro 19-4885	7.08	–	235000	0.8
Unknown	7.12	0.50	197000	

2.2. Procedures

2.2.1. Capillary electrophoresis

A Dionex Capillary Electrophoresis System I was used. A fused-silica capillary column of 75 μm inner diameter and 70 cm total length was prepared as described in the Dionex CES I user's manual [14]. Hydrodynamic injections were made by raising the sample vial containing the capillary inlet end to a height of 50 mm for 10 s. The capillary electrophoresis (CE) buffer consisted of 10 mM sodium borate and 50 mM boric acid (pH 8.6, unadjusted). Electrophoretic runs were performed by ramping the potential from 0 to 20 kV in 0.1 min and holding the potential at 20 kV (21 μA) for the remainder of the run. The capillary was rinsed with buffer and the buffer reservoirs were refilled with fresh buffer prior to each run using rinse times of 6 s, 180 s and 6 s for the destination reservoir, capillary and source reservoir, respectively.

Table 2
System precision[a] as measured by the reproducibility of the migration time and peak area of Ro 23-9424 for replicate injections of a single sample preparation

Injection number	Analyst 1		Analyst 2	
	Time (min)	Peak area	Time (min)	Peak area
1	5.99	2233	5.88	2316
2	6.03	2296	5.87	2259
3	5.94	2277	5.85	2279
4	5.94	2229	5.81	2251
5	5.95	2259	5.81	2157
6	6.02	2301	–	–
Mean	5.98	2266	5.84	2252
Std. dev.	0.04	31	0.03	59
% RSD	0.7	1.4	0.5	2.6

[a] Obtained by two analysts on different days.

2.2.2. System precision

Approximately 10 mg of Ro 23-9424 were accurately weighed into a 200 ml volumetric flask and approximately 150 ml of water were added. The solution was briefly sonicated to dissolve the solid and then diluted to volume with water. A sample of the solution was then injected onto the CE system. The solution was stored in the refrigerator and an aliquot was removed approximately 2–3 min prior to making subsequent injections. System precision determinations were performed by two analysts on different days.

2.2.3. Method precision

Six 10 mg quantities of Ro 23-9424 were weighed into separate 200 ml volumetric flasks. Approximately 150 ml of water were added. The solution was briefly sonicated to dissolve the solid, then diluted to volume with water. A sample of each solution was injected immediately after preparation. Method precision determinations were performed by two analysts on different days.

2.2.4. Linearity

Approximately 20 mg of Ro 23-9424 were accurately weighed into a 200 ml volumetric flask. Water was added and the solution was briefly sonicated to dissolve the solid. The sample was then diluted to volume with water. Appropriate aliquots of this solution were diluted to volume in a 10 ml volumetric flask to yield final concentrations ranging from 25 to 75 ppm. The samples were stored in the refrigerator at 4 °C prior to injection.

2.2.5. Limit of detection

Approximately 10 mg of Ro 23-9424 were accurately weighed into a 200 ml volumetric flask. Water was added and the solution was briefly sonicated to dissolve the solid. The sample was then diluted to volume with water. Appropriate dilutions of this solution were made in water to yield samples with concentrations of Ro 23-9424

Table 3
Method precision[a] as measured by the reproducibility of the migration time and peak area/sample weight for six sample preparations of Ro 23-9424

Ro 23-9424	Analyst 1		Analyst 2	
	Time (min)	Peak area/sample wt.	Time (min)	Peak area/sample wt.
1	6.11	217.8	6.08	226.6
2	6.03	201.3	6.03	227.2
3	6.09	210.5	6.03	221.8
4	6.07	208.0	6.04	240.3
5	6.00	204.4	5.98	233.3
6	5.96	201.7	6.02	236.4
Mean	6.04	207.3	6.03	230.9
Std. dev.	0.06	6.3	0.03	6.9
% RSD	0.9	3.0	0.5	3.0

[a] Obtained by two analysts on different days.

ranging from 5 to 0.25 ppm. The samples were stored in the refrigerator prior to injection.

2.2.6. Stability of Ro 23-9424 in water

Aliquots of a 50 ppm solution of Ro 23-9424 prepared in water (pH approximately 4.0) were injected approximately every 32 min over a 15.6 h time period.

2.2.7. Stability of Ro 23-9424 in diluent

A normal saline solution was prepared by adding 0.9 g of sodium chloride to a 100 ml volumetric flask and diluting to volume with water. A diluent solution was prepared by placing 70 mg of sodium benzoate and 70 mg of L-arginine in a 10 ml volumetric flask and diluting to volume with water. The pH of the diluent solution was approximately 11.0. A 220 mg amount of Ro 23-9424 was dissolved in 3 ml of diluent solution and 30 ml of normal saline solution with sonication. This Ro 23-9424 solution had a pH of approximately 8.7 and was stored at room temperature. At various time points, 1 ml aliquots of this preparation were placed in a 100 ml volumetric flask and diluted to volume with water immediately prior to injection. The sample preparation of Ro 23-9424 in diluent was designed to closely mimic actual drug delivery. A proposed formulation involved adding a diluent to a lyophilized cake of Ro 23-9424 for dissolution. Once dissolution was achieved, the concentrated solution was introduced via intravenous (I.V.) delivery. Stability during the duration of drug delivery was the focus of this study.

A 1 ml aliquot of Ro 23-9424 in the diluent is subdiluted to 100 ml in order to bring the concentration of the sample into the linear range of the method. Water is used to make this subdilution since the method was validated using Ro 23-9424 diluted in water. Subdiluting the sample in water prevents having to validate the method for each and every diluent which one may want to study.

3. Results and discussion

A capillary electrophoresis method was developed to separate Ro 23-9424 from its two major degradation products. The performance of this capillary electrophoresis method was evaluated with respect to specificity, system and method precision, linearity and limits of quantitation and detection. The method was then used to monitor the stability of Ro 23-9424 in water and in an L-arginine–sodium benzoate–saline diluent. The results are discussed below.

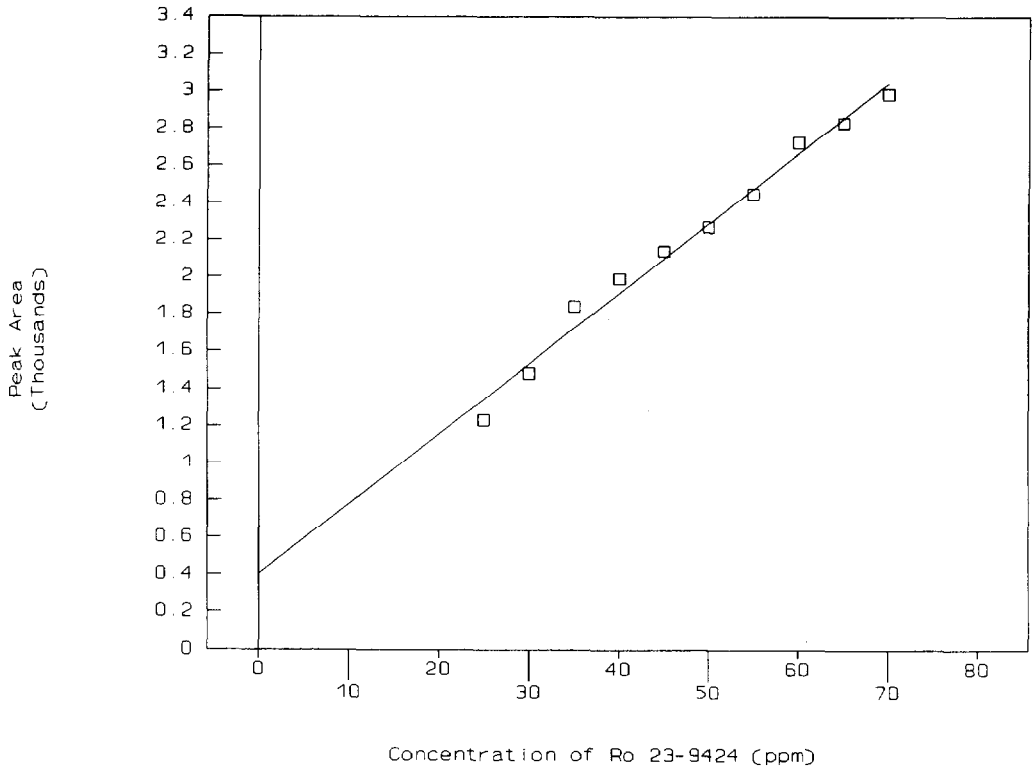


Fig. 3. Plot of peak area as a function of the concentration of Ro 23-9424.

3.1. Specificity

The separation of Ro 23-9424 and its two known degradation products, Ro 23-6240 and Ro 19-4885, are shown in Fig. 2. The identification of the peaks for Ro 23-6240 and Ro 19-4885 were made by spiking a sample of Ro 23-9424 separately with Ro 23-6240 and Ro 19-4885. This method provides separation of these components of interest in less than 8 min. In addition, an unknown impurity is observed after the Ro 19-4885 peak. The chromatographic parameters for this separation are shown in Table 1.

3.2. System precision

System precision provides a measure of system performance that is independent of the error introduced by sample handling and preparation. System precision determinations were performed by two analysts on different days by making five or six injections of a single sample preparation of Ro 23-9424. The peak area and migration times for these injections are shown in Table 2. A 0.5 and 0.7% relative standard deviation (RSD) for the migration time and a 1.4 and 2.6% RSD for the peak area was obtained. The reproducibility of the migration time and peak area is acceptable. The reproducibility of the peak area is good given the fact that Ro 23-9424 quickly hydrolyzes in solution and some degradation of Ro 23-9424 was expected during this study.

3.3. Method precision

The method precision was obtained by two analysts on different days by making injections of six sample preparations of Ro 23-9424. The migration times andn values for the peak area/sample weight for these injections are shown in Table 3. A 0.9 and 0.5% RSD for the migration times and a 3.0% RSD for the peak areas was obtained.

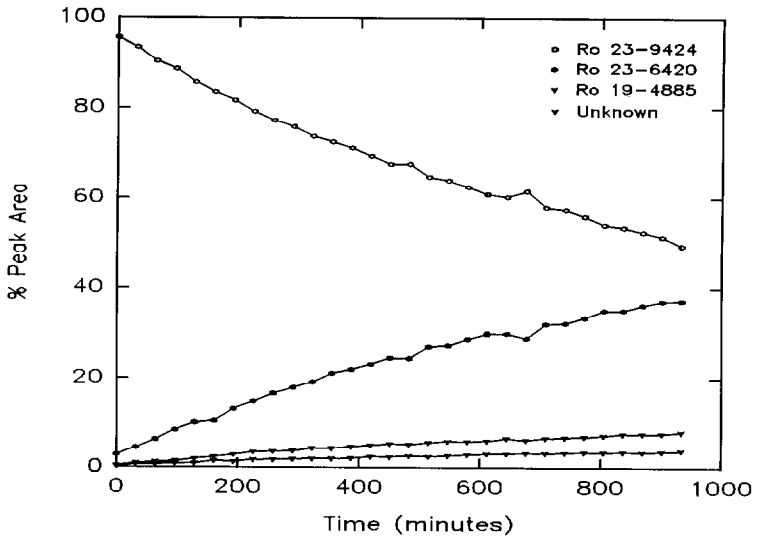


Fig. 4. Stability of Ro 23-9424 in water as a function of time. Ro 23-6240, Ro 19-4885 and an unknown were the observed degradation products.

This level of precision for the migration time is excellent and is reasonable for the peak area/sample weight.

3.4. Linearity

A plot of peak area as a function of the concentration of Ro 23-9424 is shown in Fig. 3. The concentration of Ro 23-9424 ranges from 50 to 150% of the 50 ppm working concentration of Ro 23-9424. Good linearity is observed over this range with a correlation coefficient squared, r^2, of 0.996.

3.5. Limits of detection and quantitation

The limit of detection (signal-to-noise ratio of 2) for Ro 23-9424 was determined to be approximately 0.5 ppm. This amount corresponds to a solution of concentration 6×10^{-7} M or 1% of the nominal working concentration of 50 ppm. The limit of quantitation (signal-to-noise ratio of 10) is approximately 1 ppm. These limits of detection and quantitation are sufficient for this work in which the stability of Ro 23-9424 is monitored in different formulations.

3.6. Stability of Ro 23-9424 in water and in diluent

The stability of Ro 23-9424 in water was monitored over a 15.6 h period. A plot of peak area for Ro 23-9424 and its degradation products (Ro 23-6240, Ro 19-4885 and an unknown impurity) in water as a function of time are shown in Fig. 4. The stability of Ro 23-9424 in diluent was monitored over a 24.5 h period. A plot of peak area for Ro 23-9424 and its degradation products in diluent as a function of time are shown in Fig. 5.

Ro 23-9424 hydrolyzes to form Ro 23-6240 and Ro 19-4885. Assuming a first-order reaction for this decomposition, a plot of the log of the concentration of Ro 23-9424 as a function of time yields a straight line with a slope of $-k/2.303$, where k is the rate constant. In addition, the half-life of this reaction equals $0.693/k$. Fig. 6 shows a comparison of the log of the concentration of Ro 23-9424 as a function of time in water and in diluent. The values for the slope, k and the half-life are shown in Table 4. The half-life of Ro 23-9424 in water and in diluent was calculated to be approximately 17.2 h and 34.4 h, respectively.

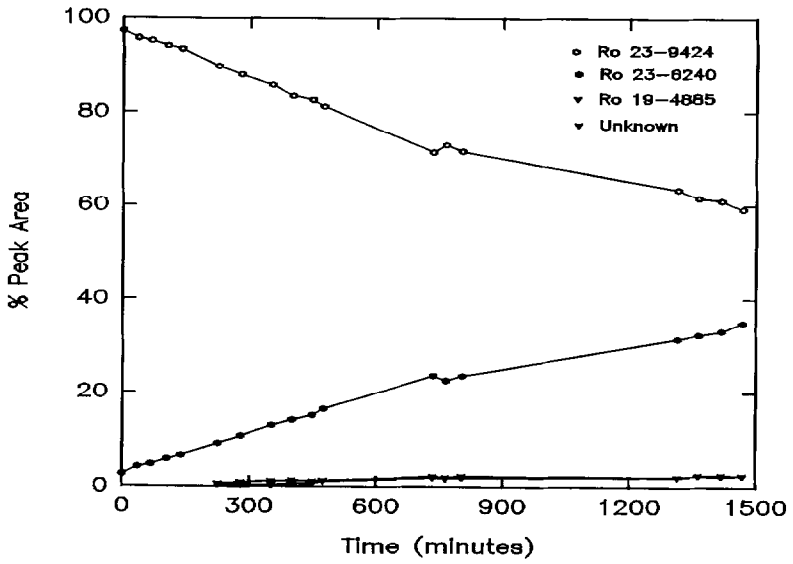


Fig. 5. Stability of Ro 23-9424 in diluent as a function of time. Ro 23-6240, Ro 19-4885 and an unknown were the observed degradation products.

The stability of Ro 23-9424 in the diluent was found to be twice that in water.

Mass balance calculations were performed for the degradation of Ro 23-9424 in water, to Ro 23-6240 and Ro 19-4885. The sum of the moles remaining Ro 23-9424 and the moles of Ro 23-6240 created during the stability study remained constant. The number of moles of Ro 23-6240 created did not equal the number of moles of Ro 19-4885 created. These calculations suggested a secondary degradation pathway for the degradation of Ro 19-4885 to an unknown impurity which was detected by capillary electrophoresis.

The number of moles of Ro 19-4885 created was subtracted from the number of moles of Ro 23-6240 created at each time point of the stability study. Then, knowing the quantity of sample injected onto the capillary, a molecular weight was calculated for each time point. The average of these molecular weights was 226.7. A proposed degradation pathway of Ro 19-4885 to form a product with a molecular weight close to 226.7 is shown in Fig. 7. This proposed degradation pathway forms degradation products with molecular weights of 230 and 201. Using a molecular weight of 230 in the calculations gave a good mass balance. A second unknown impurity possibly corresponding to a breakdown product with a molecular weight of 201 in this proposed degradation pathway was not detected in this work.

4. Conclusions

The capillary electrophoresis method developed for Ro 23-9424 separates this compound from its potential impurities, two known degradation products and one unknown degradation product. The method meets acceptable criteria for system precision, method precision and linearity. The limits of detection and quantitation for Ro 23-9424 are adequate for the scope of this work which is to compare the relative stabilities of Ro 23-9424 in two different matrices.

This work demonstrates that capillary electrophoresis is a technique which can be used to monitor the stability of a drug in different solutions as an aid in formulation method development. In this work, the stability of Ro 23-9424

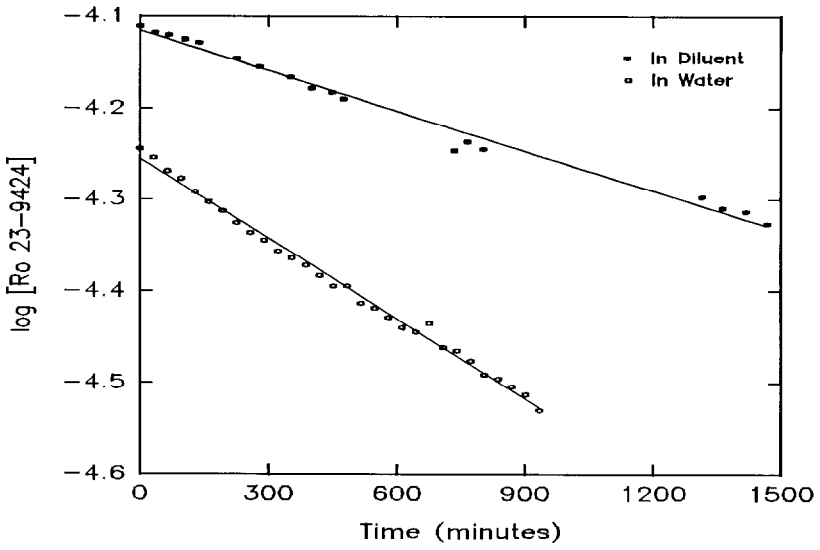


Fig. 6. Comparison of the stability of Ro 23-9424 in water and in diluent as a function of time.

was monitored in two different matrices, water and a diluent. Based on first-order kinetics, the half-life of Ro 23-9424 in diluent was calculated to be twice that in water. In addition, mass balance calculations suggested a secondary hydrolysis pathway for the degradation of Ro 23-9424.

Capillary electrophoresis is a technique that appears to be well suited to monitor the stability of compounds such as Ro 23-9424 in solution. Method development is relatively facile and easy. In this work, a method for the separation of Ro 23-9424 and its two known impurities was developed in a day. In addition, the separation of Ro 23-9424 and its impurities by capillary electrophoresis is improved compared to the separation obtained by HPLC in that the impurities are detected after the main component and not in or near the void volume.

Capillary electrophoresis, because of its stability-indicating nature and complementary separation mechanism to HPLC, should also be suitable for monitoring the purity of the drug substance itself. Typically, for pharmaceutical compounds a single chromatographic method, such as HPLC, is used to determine the purity of the compound and quantitate any impurities present in the material and a second method, such as thin layer chromatography (TLC), may also be used to quantitate any impurities. The use of a second method that is complementary to the first method increases the likelihood of detecting any unknown impurities which may be present in the material, especially if an alternate detection mechanism is used. The purity of Ro 23-9424 is currently determined by using a reversed-phase HPLC method. No TLC procedure for Ro 23-9424 exists because silica contact with the compound accelerates the

Table 4
First-order kinetic parameters (slope[a], correlation coefficient squared (r^2)[a], rate constant (k) and half-life ($t_{1/2}$)) for Ro 23-9424 in water and in diluent

Kinetic parameters	In water	In diluent
Slope	−0.0002915	−0.0001457
r^2	0.9940	0.9877
k (1/min)	0.0006713	0.0003355
$t_{1/2}$ (min)	1032	2065

[a] For the regression fit line for a plot of the log of the concentration of Ro 23-9424 vs. time (min).

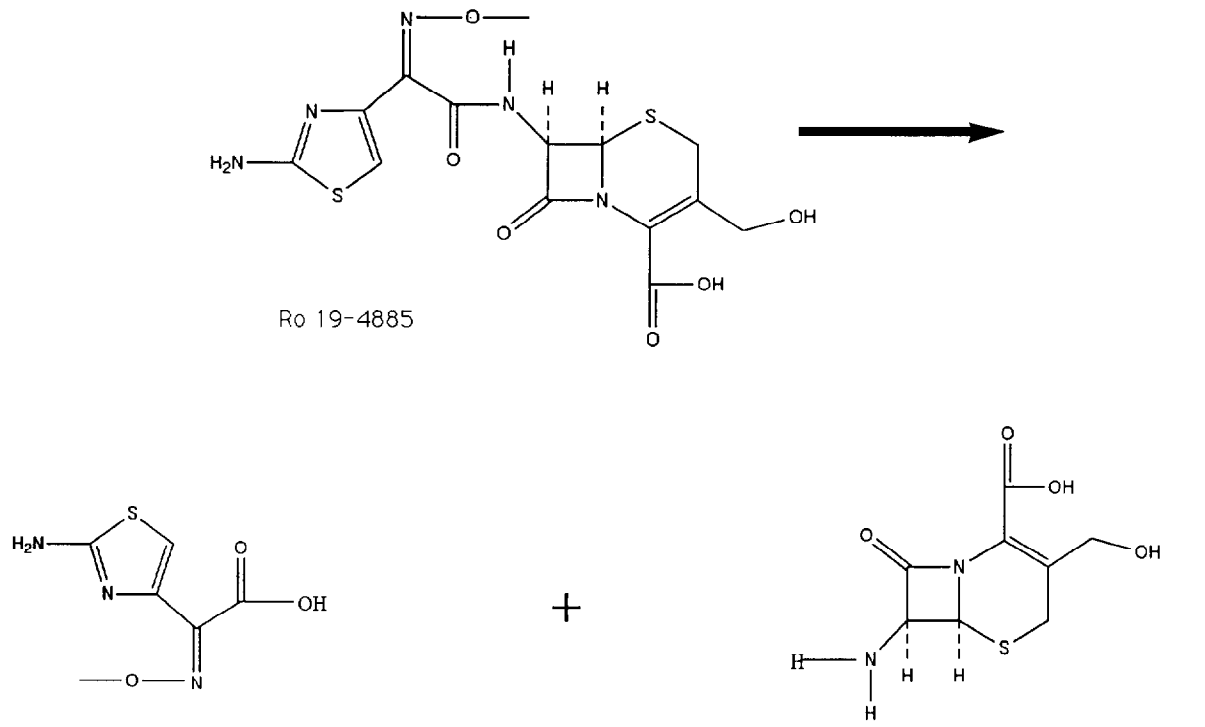


Fig. 7. Structures of the proposed degradation products of Ro 19-4885.

degradation process of Ro 23-9424. Capillary electrophoresis would appear to be a good second method for the detection of impurities in Ro 23-9424 drug substance.

The capillary electrophoresis method developed for Ro 23-9424 does have a few disadvantages. Due to the design of the capillary electrophoresis instrument used, there is no mechanism to refrigerate the samples in the carousel in order to slow the rate of hydrolysis of the analytes when analyzing a large number of samples. In addition, due to the high salt concentration of the diluent, samples had to be manually diluted prior to injection in order to avoid distortion of the electric field at the sample plug which would lead to non-gaussian peak shapes.

As in any work, the advantages and disadvantages of the use of capillary electrophoresis compared to an alternative technique need to be compared when determining whether capillary electrophoresis would be an appropriate technique for a particular project. As shown in this work, capillary electrophoresis can be well suited to monitoring the stability of a pharmaceutical compound in solution as an aid in formulation development.

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