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The determination of a degradation product in clidinium bromide drug substance by capillary electrophoresis with indirect UV detection

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Abstract

A capillary electrophoresis (CE) method utilizing indirect ultraviolet (UV) detection was developed for the determination of a non-UV absorbing degradation product, Ro 5-5172, in clidinium bromide drug substance. The electrophoresis buffer consisted of sodium phosphate and benzyltrimethylammonium bromide. Rinsing the capillary with sodium hydroxide followed by water then fresh capillary electrophoresis buffer was found to significantly improve the reproducibility of the migration times of the analytes. To further improve run-to-run reproducibility, an internal marker was used to account for differences in injection volumes and migration times between runs. The precision of the method was found to be less than 1% relative standard deviation for the migration time ratio and peak area ratio of Ro 5-5172 to the internal standard. The method was found to be linear for 0.05–1% Ro 5-5172 with respect to a 10 mg ml^{-1} sample preparation. The limit of detection was found to be less than 0.01% Ro 5-5172. Results obtained for the analysis of a clidinium bromide drug substance lot using this CE method and a thin layer chromatography method were compared and found to be in agreement. © 1997 Elsevier Science B.V.

Keywords: Degradation product determination; Drug substance; Impurity determination; Indirect UV detection; Internal standard

1. Introduction

Clidinium bromide (3-hydroxy-1-methylquinuclidinium bromide benzilate) is a quaternary ammonium compound with anticholinergic and antispasmodic activity [1]. Ro 5-5172 (3-hydroxy-1-methylquinuclidinium bromide) is one potential degradation product of clidinium bromide. This impurity is monitored in clidinium bromide drug substance and in clidinium bromide pharmaceutical dosage forms. The structures of clidinium bromide and Ro 5-5172 are shown in Fig. 1. The method currently employed to quantitiate Ro 5-5172 in clidinium bromide drug substance is thin layer chromatography (TLC) [2,3]. It was desired to evaluate alternative, more quantitative chromatographic techniques as potential replacements of TLC for quantitating this impurity. However, development of a new method for quantitating

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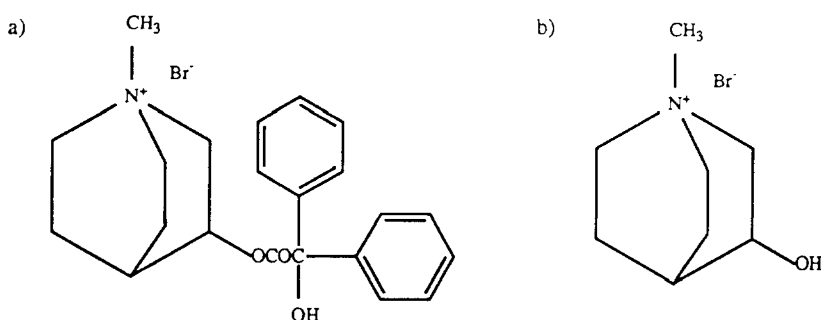

Fig. 1. Chemical structures of (a) clidinium bromide and (b) Ro 5-5172.

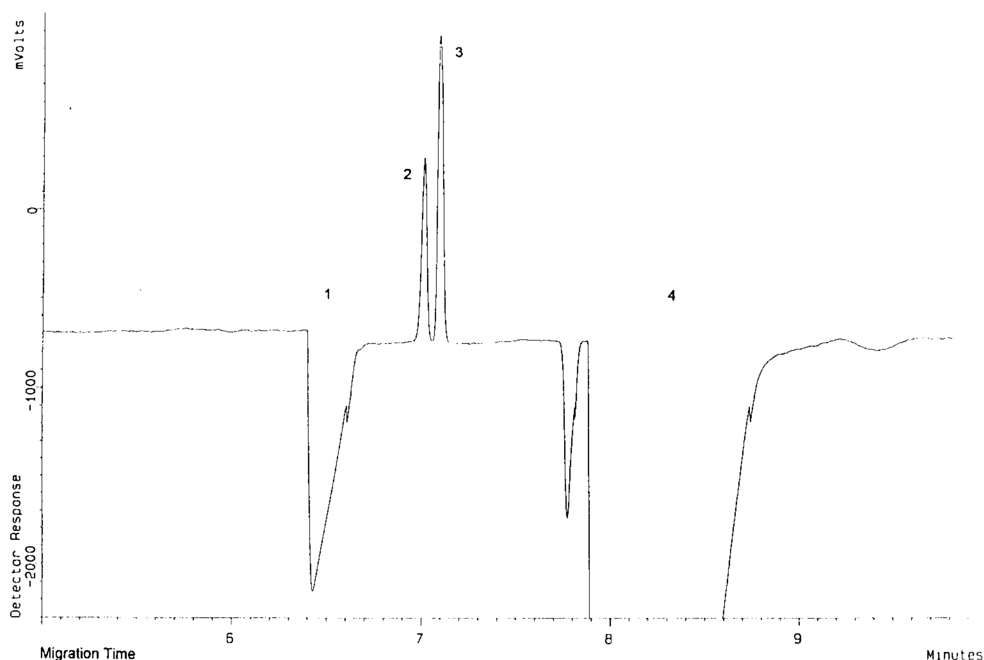

Fig. 2. Electropherogram showing the separation of the following components: (1) displaced benzyltrimethylammonium bromide; (2) Ro 5-5172; (3) internal standard; and (4) clidinium bromide.

Ro 5-5172 presented two challenging issues. One issue was whether Ro 5-5172 would have any retention by reversed-phase HPLC. A second issue was the mode of detection, since the impurity does not contain a chromophore or double bonds and therefore can not be detected by standard UV absorbance techniques.

Capillary electrophoresis (CE) with indirect UV detection was, therefore, evaluated as an alternative method to TLC for the detection of this degradation product. CE is a rapidly developing technique that has been employed in the analysis of several 'small-molecule' pharmaceuticals [4–10]. In addition, a growing number of applications on the use of CE with indirect UV detection have been reported [11–17]. The combination of CE with indirect UV detection seemed to have good potential as a technique for quantitating Ro 5-5172 in clidinium bromide drug substances. This report describes the development and validation of this method.

Table 1
Chromatographic parameters for the capillary electrophoresis separation

Component	Time (min)	Peak width (*s*)	Efficiency (*N*)	Tailing factor	Resolution ratio (*R*)
Ro 5-5172	7.19	3.0	321 000	0.88	1.64
Internal standard	7.28	3.2	296 000	1.11	

2. Experimental

2.1. Materials

Clidinium bromide and Ro 5-5172 were obtained from Hoffmann-La Roche (Nutley, NJ). Sodium phosphate monobasic was purchased from Fisher Scientific (Fair Lawn, NJ). Benzyltrimethylammonium bromide was obtained from Aldrich Chemical Company (Milwaukee, WI) and tetraethylammonium iodide was obtained from Eastman Kodak Company (Rochester, NY). All reagents and chemicals were used as received. Water was distilled and deionized using a NANOpure analytical grade system (Barnstead/Thermolyne Corporation, Dubuque, IA).

2.2. Procedures

2.2.1. Preparation of buffer

The buffer used to perform the capillary electrophoresis separations consisted of 10 mM sodium phosphate monobasic and 5 mM benzyltrimethylammonium bromide. The pH of the solution was not adjusted.

2.2.2. Preparation of internal standard solution

Approximately 50 mg of tetraethylammonium iodide was accurately weighed into a 50-ml volumetric flask. Water was used to dissolve the tetraethylammonium iodide and fill the flask to volume. The preparation was dissolved and diluted to volume with water. This solution is referred to as the internal standard solution.

2.2.3. Preparation of Ro 5-5172 standard solution

Approximately 50 mg of Ro 5-5172 was accurately weighed into a 50-ml volumetric flask. The flask was partially filled with water and sonicated briefly (<1 min) to dissolve the standard. The standard was then diluted to volume with water. This is referred to as the stock standard solution. A 0.5% standard solution was then made by pipetting 5 ml of stock standard solution into a 100-ml volumetric flask. Then, 10 ml of internal standard solution was added. The preparation was diluted to volume with water. The final concentration of this standard solution was 0.5% Ro 5-5172 (0.05 mg ml^{-1}) and 100 ppm internal standard.

2.2.4. Preparation of clidinium bromide sample

Approximately 100 mg of clidinium bromide was accurately weighed into a 10-ml volumetric flask and 1 ml of internal standard solution was added. The volumetric flask was partially filled with water and sonicated briefly (<1 min) to dissolve the sample. The sample was then diluted to volume with water. The sample solution was injected immediately after preparation.

2.2.5. Capillary electrophoresis

Capillary electrophoresis separations were performed using a Dionex Capillary Electrophoresis System I (Model CES-I). The capillary used had an inner diameter of 75 μm, a total length of 70 cm and was prepared as described in the Dionex CES-I manual [18]. The capillary was rinsed with the following solutions between runs using 10 psi for 300 s: 1 N sodium hydroxide; water; and electrophoresis buffer. After these rinses were complete, the buffer was replaced in the destination reservoir, source reservoir and capillary using 10 psi for 6, 180 and 6 s, respectively. Hydrodynamic injections were made at 50 mm for 10 s (approximately 6 nl injection volume). Separations were performed at ambient temperature using 15 kV (12 μA) and indirect UV detection was performed at 205 nm. The polarity of the signal

Table 2
Migration time, peak area and ratio data for the injection of a 0.05 mg ml^{-1} standard solution

Injection	Time (min)	Area	Ratio of time[a]	Ratio of area[b]
1	7.16	2739	0.988	0.691
2	7.19	2750	0.988	0.700
3	7.14	2721	0.989	0.693
4	7.18	2758	0.989	0.699
5	7.16	2715	0.989	0.694
6	7.11	2671	0.989	0.689
Mean	7.16	2726	0.989	0.694
%R.S.D.	0.4	1.2	0.07	0.61

[a]Ratio of the migration time of Ro 5-5172 to the migration time of the internal standard.
[b]Ratio of the peak area of Ro 5-5172 to the peak area of the internal standard.

going to the data acquisition system was reversed so that the Ro 5-5172 peak was positive.

3. Results and discussion

A CE method was developed to quantitate the level of Ro 5-5172 in clidinium bromide drug substances. Since Ro 5-5172 does not contain a chromophore or double bonds, indirect UV detection at 205 nm was used. Indirect UV detection was performed by placing a UV absorber, benzyltrimethylammonium bromide, in the capillary electrophoresis buffer. The presence of the UV absorber in the buffer results in a high UV absorbance background during the electrophoretic run. In solution, the UV absorber exists as the benzyltrimethylammonium cation and the bromide anion. The positively charged ion of Ro 5-5172 in the sample solution will displace benzyltrimethylammonium cations in the buffer by charge displacement [19,20], and result in less absorbance, or a negative peak, at the Ro 5-5172 sample band in the capillary. The displaced benzyltrimethylammonium cations will be detected as a positive peak. Clidinium bromide itself does absorb so it will be detected as a positive peak. The internal standard, not a UV absorber, is also detected as a negative peak. Since the data acquisition system used in this work could not integrate negative peaks, the polarity of the signal going to the data acquisition system was reversed. In this way, Ro 5-5172 and the internal standard were detected as positive peaks, while clidinium bromide and the displaced benzyltrimethylammonium cations were detected as negative peaks.

The migration times of Ro 5-5172 and the internal standard were initially found to be irreproducible. The migration time would continually increase with successive runs. This phenomena was believed to be due to the interaction of the positively charged benzyltrimethylammonium cations with the negatively charged silanol groups on the surface of the capillary. This interaction would cause a time dependent decrease in the negative charge of the capillary surface and hence cause a decrease in the electroosmotic flow over time. This would lead to longer and longer migration times for the analytes. Rinsing the capillary with sodium hydroxide followed by water, then fresh capillary electrophoresis buffer was found to significantly improve the reproducibility of the analyte migration times. Sodium hydroxide apparently strips the benzyltrimethylammonium cations from the surface of the capillary and regenerates the negatively charged silanol groups.

In an effort to further improve run-to-run reproducibility, triethylammonium iodide was used as an internal standard to account for differences in injection volumes between runs and for differences in migration times between runs. By taking a ratio of the migration time and peak area of Ro 5-5172 to the migration time and peak area of this internal standard, the reproducibility in terms of %R.S.D. for a given number of runs was further improved.

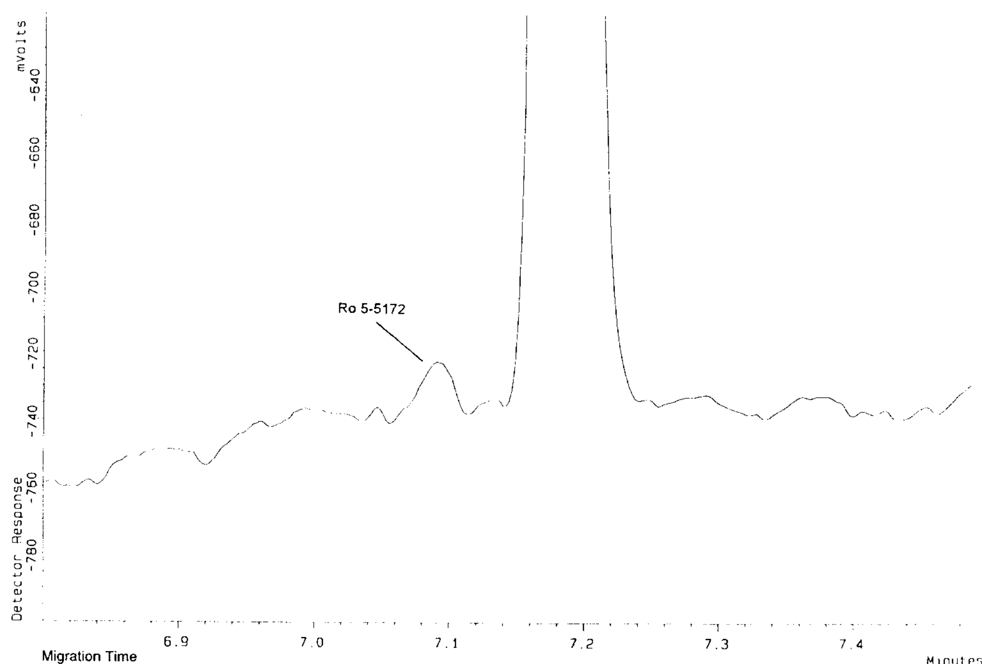

Fig. 3. Electropherogram of 0.78 μg ml^{-1} Ro 5-5172.

The performance of the CE method was evaluated with respect to specificity, system precision, linearity and limit of detection. A comparison of results obtained for the analysis of a batch of clidinium bromide drug substance, by using CE and TLC, was made. The results of these studies are discussed below.

3.1. Specificity

The CE method separates Ro 5-5172, clidinium bromide and tetraethylammonium iodide, the internal standard. An electropherogram showing the separation of Ro 5-5172, the internal standard and clidinium bromide is shown in Fig. 2. The relevant chromatographic parameters for Ro 5-5172 and the internal standard, calculated according to USP 23 [21], are listed in Table 1. Clidinium bromide and the displaced benzyltrimethyl ammonium bromide peak were detected as negative peaks and therefore could not be integrated.

The negative peak which is detected in front of clidinium bromide may be a UV absorbing impurity of clidinium bromide. The identification of this peak, however, was not attempted since the focus of this work was to develop a method to quantitate Ro 5-5172.

3.2. System precision

System precision allows the evaluation of the reproducibility of the separation method irrespective of sample preparation and handling. Six injections of a single preparation of a 0.5% (0.05 mg ml^{-1}) standard solution of Ro 5-5172 with internal standard were made. A 0.4% R.S.D. for migration time and a 1.2% R.S.D. for peak area were obtained for Ro 5-5172. The reproducibility is improved by taking a ratio of the migration times and peak areas for Ro 5-5172 to those of the internal standard. In this way, a 0.07% R.S.D. for the migration time ratio and a 0.61% R.S.D. for the peak area ratio were obtained. These values are summarized in Table 2. As the data demonstrate the reproducibility of the CE method is improved by using migration time and peak area ratios.

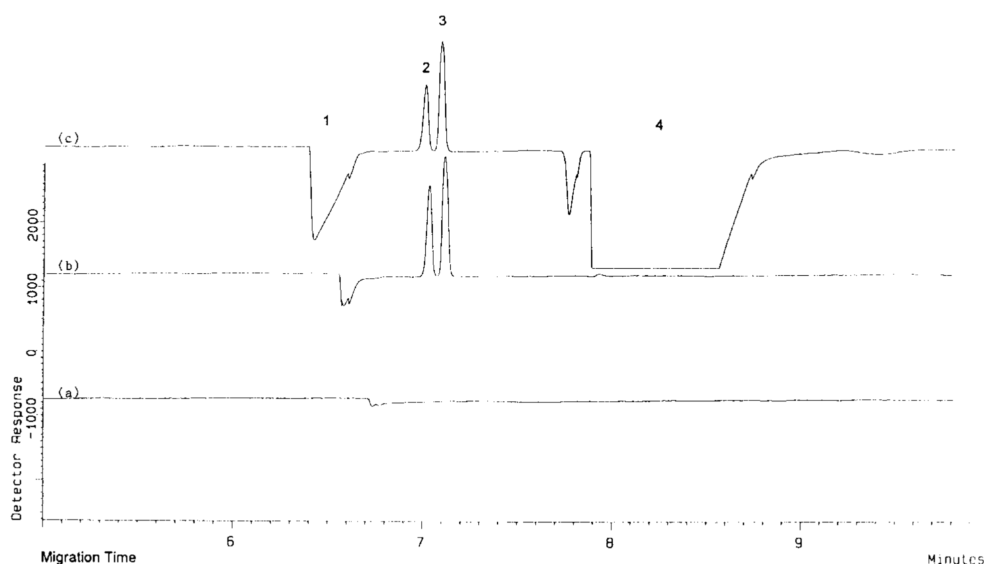

Fig. 4. Electropherograms for (a) blank (water), (b) standard solution (Ro 5-5172 and internal standard) and (c) clidinium bromide drug substance sample. Peak identities are as follows: (1) displaced benzyltrimethylammonium bromide; (2) Ro 5-5172; (3) internal standard; and (4) clidinium bromide.

3.3. Linearity

The linearity of the method was evaluated from 0.005 to 0.10 mg ml^{-1} Ro 5-5172. This represents from 0.05 to 1% Ro 5-5172 with respect to a 10 mg ml^{-1} clidinium bromide sample preparation. Regression analysis of the ratio of the Ro 5-5172 peak area to the peak area of the internal standard resulted in a correlation coefficient of 0.9946 with an intercept which included the origin (95% confidence interval). This linearity range of Ro 5-5172 is suitable for the quantitation of this potential impurity in clidinium bromide drug substance since the control limit of Ro 5-5172 is 0.5%.

3.4. Limit of detection

An electropherogram for the injection of 0.78 μg ml^{-1} Ro 5-5172 is shown in Fig. 3. This concentration represents 0.0078% Ro 5-5172 with respect to a 10 mg ml^{-1} clidinium bromide sample preparation. At this concentration the signal-to-noise ratio is approximately 2, based on the noise level in the 7.3–7.5 min range. This concentration therefore, corresponds to the limit of detection. An approximate limit of quantitation, based on a signal-to-noise ratio of 10, would therefore be predicted to be approximately 3.9 μg ml^{-1} or 0.039% Ro 5-5172. The control limit for Ro 5-5172 is 0.5% so a limit of detection of less than 0.01% and a limit of quantitation of approximately 0.04% is acceptable for determination of this impurity in clidinium bromide drug substance.

3.5. Analysis of clidinium bromide drug substance

Analysis of a batch of clidinium bromide drug substance indicated the presence of 0.44% Ro 5-5172. This result is in good agreement with TLC analysis of the same batch which found between 0.4 and 0.45% Ro 5-5172. A plot of the electropherograms for the sample, a standard and a blank are shown in Fig. 4. The good agreement in results obtained by using CE and TLC shows that CE with indirect UV detection is a suitable technique for the determination of Ro 5-5172 in clidinium bromide drug substance. The method was not attempted with the dosage form.

4. Conclusions

A capillary electrophoresis method with indirect UV detection was developed and evaluated for the determination of a degradation product, Ro 5-5172, of clidinium bromide drug substance. The method developed is a suitable alternative to the currently used TLC procedure. The method was found to have acceptable specificity, linearity and limit of detection for the impurity of interest. Excellent system precision (0.07% R.S.D. for migration time ratios and 0.61% R.S.D. for peak area ratios) was obtained by rinsing the capillary with sodium hydroxide between runs and by using an internal standard. In addition, CE offers the advantage of being quantitative and automated. This work demonstrates that CE with indirect detection is a viable technique for the detection and quantitation of non-UV absorbing analytes.

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