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Capillary electrophoresis determination of loratadine and related impurities

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Abstract

While HPLC has traditionally been the method of choice for purity determination of pharmaceutical substances, capillary electrophoresis (CE) offers a different selectivity and hence it is a complementary technique to HPLC. Loratadine, an antihistamine, could include in its raw material seven impurities that ought to be separated, identified and quantified for drug development and quality control. As a complementary tool for undoubtful identification, a CE method has been developed. The separation was carried out with an uncoated fused-silica capillary (57 cm × 50 μm ID) and was operated at 20 kV potential. Temperature was maintained at 25 °C. The final separation buffer was prepared with 100 mM H_3PO_4 made up to pH 2.5 with NaOH and with 10% acetonitrile added (v/v). Impurities can be detected at the 0.1% level of the active and validation parameters for linearity accuracy and precision are adequate for all the analytes and that permits to consider the method reliable and suitable for application to long-term stability and purity studies.

Keywords: Loratadine; Impurities; Capillary electrophoresis

1. Introduction

Capillary electrophoresis (CE) is now an established technique in several areas of analysis. Pharmaceutical companies make extensive use of CE, in particular for chiral separations and the technique is widely accepted by regulatory authorities such as FDA. However, inexperience of CE and the predominance of HPLC in many analytical laboratories continue to impede uptake of the technique. This is despite the fact that for many analyses CE may be easier, faster and more cost-effective due to the low solvent consumption and the use of cheap capillaries. On the other hand, impurity profiling is an important issue in pharmaceutical analysis, particularly during product development, quality control and long-term stability tests. This is because of their differences with respect to selectivity; HPLC and CE are often complementary and it may be of great interest for the impurities determination to develop these two techniques simultaneously.

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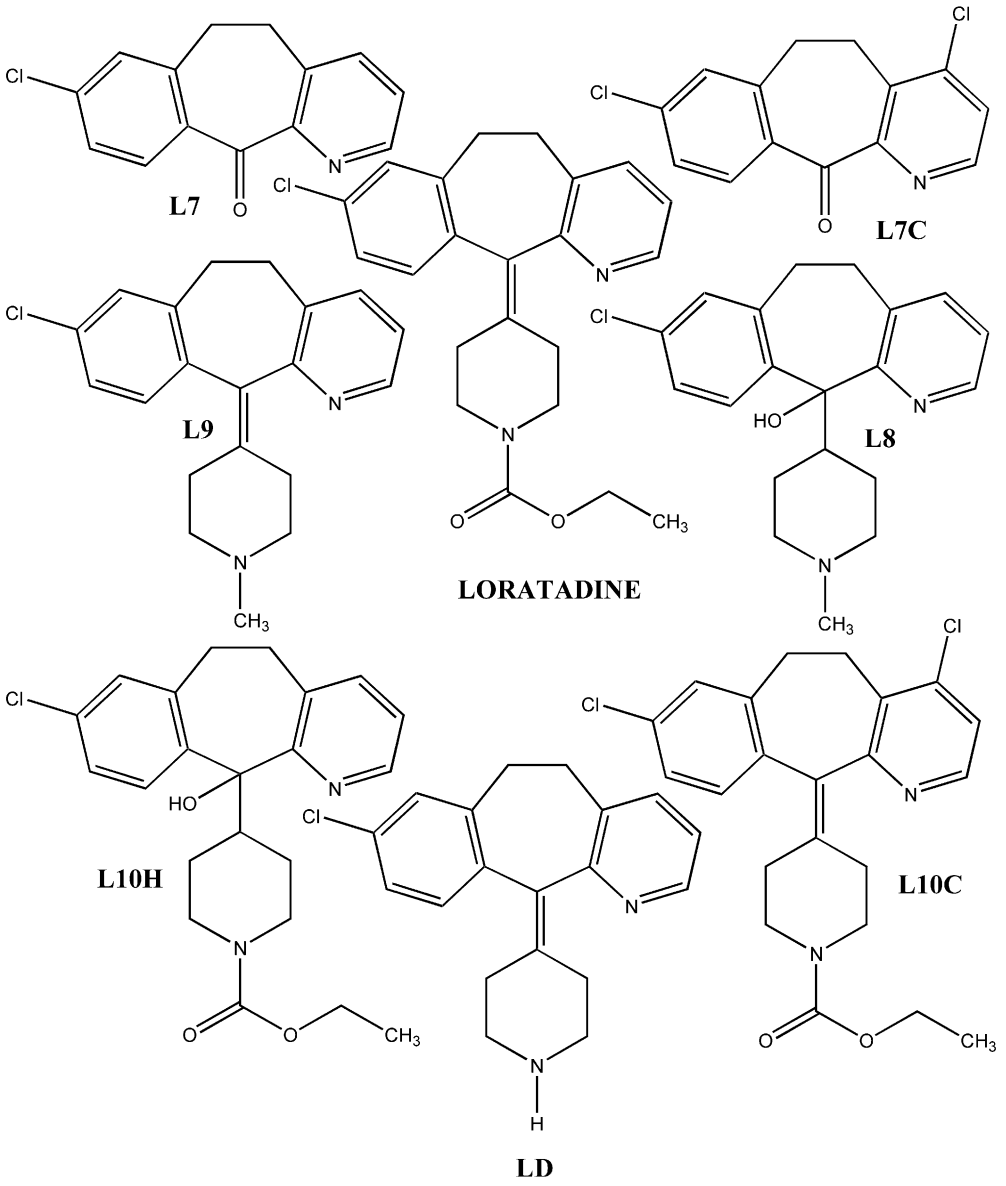

Fig. 1. Chemical structures of loratadine and related impurities.

Loratadine is a long-acting tricyclic antihistamine with selective peripheral histamine H1-receptor antagonistic activity. Loratadine is a white powder not soluble in water, but very soluble in organic solvents. Its chemical name is ethyl-4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidine carboxylate. It is possible that bulk loratadine could include seven impurities that ought to be separated, identified and quantified in its analysis. Its formula and

those corresponding to its impurities can be seen in Fig. 1, where it can be observed that the main component and structurally related impurities posses in most of them similar structures and thus physicochemical properties, which make the resolution difficult.

Few analytical methods have been described for loratadine, most of them have been developed for pharmacokinetic studies and they are applied to quantify loratadine and its metabolite descarboethoxyloratadine in plasma by HPLC [1], GC–MS [2], and GC with N–P detector [3].

Four methods have been described for loratadine assay in pharmaceutical preparations: a polarographic method [4], which requires previous derivatisation, a spectrophotometric and an HPLC method [5], for the main component assay and only one method, recently developed in our laboratory, permits impurity evaluation [6].

In CE, the selectivity of the method is fundamentally based on charge-to-volume ratios. Therefore, CE can be an "orthogonal" procedure with totally different selectivity.

The standard requirements of such an impurity method is that all likely synthetic and degradative impurities are resolved from each other and the main drug and that impurities can be monitored at the 0.1% level or below.

The aim of this paper was the development and validation of a CE method for the identification and quantification of loratadine and its related impurities in raw material and in tablets as pharmaceutical presentation.

2. Experimental

2.1. Apparatus

The separation was performed on a CE P/ACE 5500 (Beckman) with UV detection at 200 nm. The injection was by pressure (3.3 bar) for 5 s. The separation was carried out with an uncoated fused-silica capillary (57 cm × 50 μm ID) and was operated at 20 kV potential. Temperature was maintained at 25 °C. The final separation buffer was prepared with 100 mM H_3PO_4 made up to pH 2.5 with NaOH and with 10% acetonitrile added (v/v).

2.2. Chemicals

Standards of loratadine and impurities as well as tablets and placebo of the speciality were kindly provided by CINFA S.A. (Pamplona, Spain).

Phosphoric acid 85% was from Merck (Darmstadt, Germany) and NaOH and the other organic solvents were HPLC grade from Scharlab (Barcelona, Spain).

2.3. Optimisation of CE method

Selectivity in CZE can be controlled by background electrolyte (BGE) concentration, pH, organic modifiers and capillary length. All these parameters were varied and results are summarised below.

2.4. Standard solutions and sample preparation

Loratadine stock solution was prepared with 521.7 mg of loratadine exactly weighed and dissolved in a 25 ml volumetric flask with acetonitrile. Stock solutions of every impurity were individually prepared with 10 mg exactly weighed and dissolved in 25 ml volumetric flasks with acetonitrile. For quantitation, 178.4 mg of the pulverised tablets were made up to 25 ml, with acetonitrile/5 mM phosphate buffer at pH 3.0, 60:40 (v/v), after waterbath sonication for around 10 min samples were filtered with 0.45 μm nylon filters prior to the injection. The standard was prepared with 0.8 ml of stock solution of loratadine made up to 25 ml with the same dissolution medium. When impurities ought to be measured, the standard was prepared in 25 ml volumetric flasks containing 0.8 ml of loratadine stock solution with 42 μl each of the stock impurities solution, plus acetonitrile to complete 15 ml and they were levelled off with 5 mM phosphate buffer at pH 3.0.

2.5. Validation

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to present. Typically these might include impurities, degradants, excipients, etc. During validation, the discrimination of the analyte in the presence of impurities and/or excipients tested specificity. It was done by injecting the placebo of the pharmaceutical speciality and checking that there was no interfering peak and by spiking the drug substance and the drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Moreover, identification of each impurity was confirmed with migration time as compared with those of pure standards and by spiking.

The linearity study verifies that the sample solutions are in a concentration range where analyte response is linearly proportional to concentration. For main component assay methods, this study is generally performed by preparing standard solutions at five concentration levels from 50 to 150% of the target analyte concentration. In this case, loratadine concentrations were from 0.333 to 1.000 mg/ml. They were prepared in 25 ml volumetric flasks with 0.4, 0.6, 0.8, 1.0 and 1.2 ml of stock loratadine solution, plus acetonitrile to complete 15 ml and 5 mM phosphate buffer at pH 3.0 to make the volumes. Each point was analysed three times. For sample linearity, five solutions were identically prepared but with the proportion of the excipients of the speciality (161.7 mg) added to each flask.

For impurity methods, linearity is determined by preparing standard solutions at five concentration levels over a range such as 0.05–1.0 wt.%. In this case, standards ranged from 0.3 to 1.0 μg/ml and they were prepared in 25 ml volumetric flasks containing 0.8 ml of loratadine stock solution with 21, 32, 42, 53, 63 and 74 μl of the intermediate impurities solution, plus acetonitrile to complete 15 ml and they were leveled-off with 5 mM phosphate buffer at pH 3.0. Sample linearity was tested in the same way but with the proportional weight of the excipients of the speciality (161.7 mg) added to each flask.

The accuracy of a method is the closeness of the measured value to the true value for the sample. For pharmaceutical studies, the most widely used approach is the recovery study which is performed by spiking analyte in blank matrices. It was tested in the same linearity assay for both main component and impurities. The percent recovery and RSDs were then calculated.

The precision of an analytical method is the amount of scatter in the results obtained from multiple analyses of a homogeneous sample. The first type is repeatability or intra-assay precision. Intra-assay precision data were obtained by repeatedly analysing, in one laboratory on 1 day, six aliquots of a homogeneous sample, each of which were independently prepared according to the method procedure. The second type is intermediate precision. These data were obtained by repeating the intra-assay experiment on a different day with newly prepared mobile phase and samples.

The detection limit of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically, three times the noise level. The detection limit needs to be determined only for impurity methods in which chromatographic peaks near the detection limit will be observed.

The quantitation limit is the lowest level of analyte that can be accurately and precisely measured. This limit is required only for impurity methods and the best option is to have it determined by reducing the analyte concentration until a level is reached where the precision of the method is unacceptable. As a theoretical approach, that ought to be checked, the quantitation limit is often calculated as the analyte concentration that gives $S/N = 10$.

3. Results and discussion

The dissolution medium for samples was a compromise between the low solubility of loratadine in polar media, although increased at acidic pH, and the possibility of current losses with a high proportion of organic solvent. Finally, it was

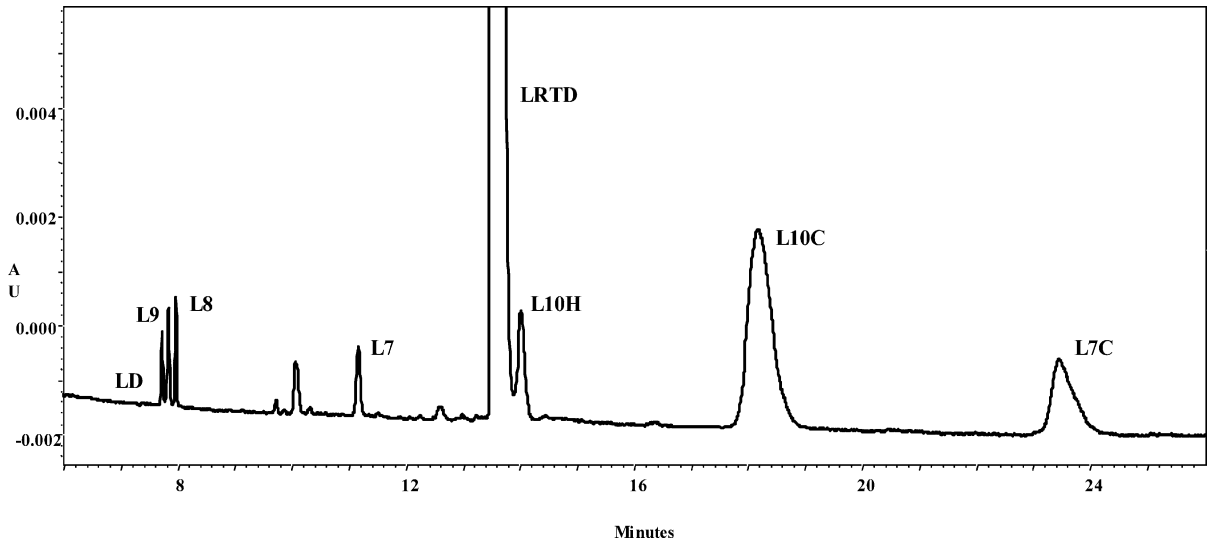

Fig. 2. Electropherogram showing loratadine (0.6 mg/ml) and related impurities (all of them 0.1% of the main peak) separation. The buffer consisted of H_3PO_4 100 mM (H_2O) brought up to pH 2.50 with NaOH and 10% acetonitrile (v/v). UV detection: 200 nm.

acetonitrile/5 mM phosphate buffer at pH 3.0, 60:40 (v/v).

For the BGE, phosphate was chosen mainly due to its buffer capacity near pH 2.5 and because it does not absorb at 200 nm. Although loratadine presents a characteristic absorbance maximum at 240 nm, the signal is higher at 200 nm and this wavelength is critical for detecting the impurities under the 0.1% level. That is also a point for choosing zonal CE instead of another mode of electrophoresis with noisier baselines at 200 nm. Buffer concentrations ranging from 50 to 200 mM phosphate were tested and, as it was expected, higher buffer concentrations increased migration times, but also the peak width, providing poorer limits of detection for the two last impurities. Finally, 100 mM phosphate was the best option. pH varied from 2.50 to 4.00 with increments of 0.25 units. Migration time increased noticeably with increasing pH for the last two impurities, which present a group with lower basicity due to the chloro in the pyridine ring and poorer resolution was also obtained for the three impurities at the beginning of the electropherogram, therefore pH 2.50 was selected. Different compounds such as NH_3 and triethylamine besides NaOH were tested for adjusting pH in order to avoid the possible adsorption of the compounds in the capillary wall. None of them gave the expected results; moreover, loratadine did not elute from the capillary in many cases. Therefore, pH was adjusted with NaOH. Finally, since during prevalidation assays some variations were observed in loratadine peak areas and irregularities in the current, probably due to its insolubility in the aqueous media of the BGE, organic modifiers such as methanol and acetonitrile were added. They ranged from 5 to 20%. Ten percent of acetonitrile 10% (v/v) in BGE gave the best result regarding to resolution and peak shape.

Peaks were identified with their migration time related to a pure standard of the same compound and by spiking, because UV spectra of all the analytes are very similar. Finally, the electropherogram obtained in the optimised condition can be seen in Fig. 2.

Migration time order increased in good approximation with the expected charge to mass ratios at the working pH, being the compounds with a chloro in para of the pyridine ring less basic and,

Table 1
Main validation parameters for loratadine and related impurities by CE (CI: confidence interval)

Parameter	LD	L8	L9	L7	Loratadine	L10H	L10C	L7C
Linearity								
Range (μg/ml)	0.366 – 1.099	0.353 – 1.058	0.363 – 1.089	0.383 – 1.149	333.3 – 1000	0.356 – 1.069	0.329 – 0.099	0.369 – 1.108
Standards								
Intercept ± CI	−353 ± 874	−2619 ± 1195	−294 ± 894	−3366 ± 2151	−182940 ± 2890577	−5931 ± 3245	−19870 ± 8638	321 ± 2029
Slope ± CI	8738940 ± 1151759	14436530 ± 1804952	9799968 ± 1189883	20863628 ± 2598562	3774728 ± 378145	22761715 ± 4611238	4354497 ± 12088083	20344186 ± 2619277
r	0.99	0.99	0.99	0.97	0.99	0.97	0.99	0.98
Samples								
Intercept ± CI	−2639 ± 1248	−1469 ± 1269	711 ± 1250	−2246 ± 2049	448211 ± 21680	372 ± 2082	4177 ± 8156	2730 ± 6446
Slope ± CI	13917131 ± 1894445	12527024 ± 1999424	7769449 ± 1655589	17445648 ± 2598417	3228588 ± 302532	11422191 ± 2554975	22568661 ± 8721563	18504399 ± 7717989
r	0.99	0.98	0.98	0.98	0.99	0.98	0.99	0.95
Accuracy								
Samples								
Recovery (%)	110	102	97	93	100	95	84	111
RSD (%)	11	9	12	9	7	19	15	10
Precision								
Intra-assay								
N	6	6	6	6	6	6	6	6
Mean (μg/ml)	0.64	0.62	0.63	0.71	676.7	0.75	0.69	0.72
RSD (%)	4	3	7	10	6	11	6	5
Intermediate								
N	12	12	12	12	12	12	12	12
Mean (μg/ml)	0.72	0.67	0.70	0.73	666.9	0.70	0.69	0.74
RSD (%)	10	12	15	10	6	11	7	10

therefore, with less charge, the last to elute. Neither loratadine nor its impurities interfered with the excipients of the speciality which were those typically contained in tablets (lactose, starch, magnesium stearate and polyvinylpyrrolidone).

After development, experiments for evaluating the validity of the method for determining loratadine and related impurities in raw material and in tablets were carried out. The main validation parameters of the method are shown in Table 1.

One of the major weak points of CE is its poor precision [7] and that is reflected in validation results. Reasonable linearity and accuracy have been obtained for all the analytes, but the variations in the responses provided some parameters, which did not comply the expected values. Since imprecision in CE is usually attributed to the problems related with the injection of nanolitre sample volumes and with evaporation of the sample solvent when it has a high organic solvent content, fluoxetine was tested as an internal standard. It gave a narrow and good shaped peak near loratadine, nevertheless, did not improve the results and, therefore, it was not employed. Since the temperature was well maintained by the equipment, the buffer was frequently changed. The current was stable and the internal standard did not improve the results, analyte–wall interaction seemed to be the major cause for area variation, but the general strategies adopted to solve problems caused by adsorption and fluctuations of the electro-osmotic flow [7] did not improve the results either.

Limits of quantification must be established with the lower concentrations in which the method can be validated with enough precision and accuracy. Experimental limits of quantification were established in 0.05% (0.3 μg/ml) for the seven impurities. These limits are the lowest concentration values of the impurities measured in the validation and passing the acceptance criteria. Therefore, they are more reliable than the values obtained with mathematical approaches, because all the impurities and the parent compound are present in the same run.

Calculated limits of quantification were determined for loratadine, just to compare with the experimental values. The method employed was EURACHEM [8] for which six replicates of five points in the lower range (0.05–0.25 wt.%) were measured. LOQ is established by representing concentration versus RSD and interpolating the concentration corresponding to 10% of RSD. The result obtained was 0.46 μg/ml which is quite near the experimental value. The mathematical estimation of the limits of confidence corresponded, therefore, to 0.14 μg/ml.

As an indication of the robustness of the method, small variation in the pH of the buffer (± 0.1) and different capillaries were tested without change in resolution. The stability of the standards had been previously tested in our previous work that was over 1 week in solution at 4 °C [6].

4. Conclusion

A CE method has been developed for loratadine and related impurities identification and measurement in raw material and tablets. The advantages of this new method are those related with CE, as for example, the low consumption of reactives. The main contribution of the method is that it can be a complementary tool for impurity profiling during stability tests. Nevertheless, validation parameters of the method are poorer than those described for HPLC [6] for the same compounds and therefore, in this case, HPLC would be the preferably tool for quantitation.

Acknowledgements

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