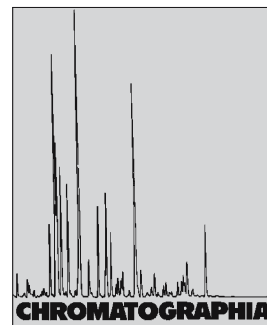


LC Determination of Lercanidipine and Its Impurities Using Drylab Software and Experimental Design Procedures



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Abstract

The main objective in all optimization procedures is to define the most appropriate conditions for rapid, sensitive, precise, and reproducible analysis, as economically as possible. Experimental design and DryLab optimization software have been used to optimize a liquid chromatographic method for separation of lercanidipine and its three impurities. In both methods of optimization the acetonitrile content and pH of the mobile phase were factors extracted for analysis; resolution of a critical pair was output in both cases. Data obtained from both optimization methods were compared and appropriate conclusions were extracted with the objective of gaining a complete view of chromatographic behavior. Detailed description was obtained by use of a three-dimensional graph and DryLab maps. Both methods of optimization reported the most appropriate mobile phase to be a 35:65 mixture of acetonitrile and an aqueous solution of 1.5% TEA, pH adjusted to 3.0 by addition of orthophosphoric acid, at a flow rate 1.0 mL min⁻¹. Separations were performed on a 20 × 4.6 mm, 3.5-μm particle size, C₁₈ column, at 20 °C, with UV detection at 240 nm. The method was validated. All the results proved the reliability of the method, so it can be used for separation, identification, and simultaneous determination of these substances in the drug and in pharmaceutical dosage forms.

Keywords

Column liquid chromatography
Optimization
Experimental design
DryLab software
Lercanidipine hydrochloride
Impurities
Validation

Introduction

Liquid chromatographic methods for assay of active pharmaceutical ingredients (API) and impurities in drug products must meet stringent regulatory requirements, must be transferable globally to different units, and must remain operational over the lifetime of the product. They should not be too complicated and time-consuming to run, and should be as cost-effective as possible. The first stage of chromatographic method development is to identify the most promising column, buffer or aqueous phase, and organic solvent that will be used to separate the API and its impurities in a particular sample. When initial starting conditions have been found, the next stage is to find the optimum separation conditions. This task becomes more complicated as the number of operating variables increases, so a larger number of experimental runs is required. To simplify and accelerate the optimization process, several computer simulation software packages have been introduced [1, 2]. With a limited number of experimental runs, chromatographers can model changes in experimental conditions, optimize method conditions, and predict method robustness [3–5]. Software strategies have gained widespread

Standard Solutions for Linearity Testing

Stock solutions were prepared by dissolving the working standards in mobile phase; the concentrations were 1.0 mg mL⁻¹ for lercanidipine hydrochloride, and 3.0 µg mL⁻¹ for impurities B, 1, and 3. For construction of calibration plots ten solutions were prepared in the concentration ranges 0.07–1.0 mg mL⁻¹ for lercanidipine hydrochloride and 0.21–3.0 µg mL⁻¹ for impurities B, 1, and 3. Solutions of the active compound and the impurities were prepared protected from light and used within two hours of preparation to prevent degradation.

Solutions for Accuracy and Precision Testing

To validate the proposed RP-HPLC method, solutions of lercanidipine hydrochloride and impurities B, 1, and 3 were prepared in the ratio of the amounts present in tablets.

The accuracy of the method was evaluated in triplicate for three concentrations of lercanidipine hydrochloride—80% (0.4 mg mL⁻¹), 100% (0.5 mg mL⁻¹), and 120% (0.6 mg mL⁻¹). Recovery (%) was calculated from the slope and *y* intercept of the calibration plot obtained. The accuracy of measurement of the impurities was determined by triplicate analysis of 1.2, 1.5, and 1.8 µg mL⁻¹ solutions. Recovery (%) was calculated from the slopes and *y* intercepts of the calibration plots obtained.

The precision of the method was evaluated by triplicate analysis of ten solutions of lercanidipine hydrochloride at each of the three concentrations 0.40, 0.50, and 0.60 mg mL⁻¹ against a qualified reference standard and the RSD (%) of the assay was calculated. The precision of the related substance method was checked by triplicate injection of ten solutions of each of impurities B, 1, and 3 at each of the three concentrations 1.2, 1.5, and 1.8 µg mL⁻¹. RSD (%)

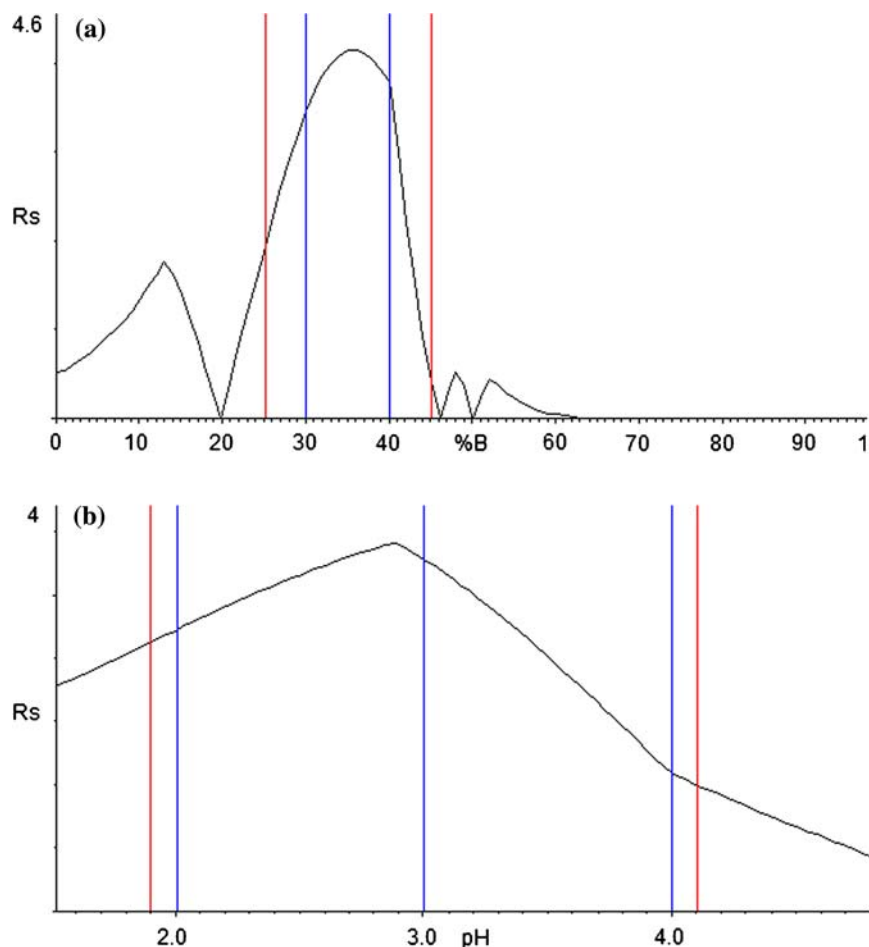


Fig. 2. Resolution map. Dependence of the resolution of the critical pair impurity B and impurity 1 on: (a) the ACN content of the mobile phase, at pH 2, and (b) the pH of mobile phase containing 35% (v/v) ACN

of peak area was calculated for each impurity.

Solutions of the active compound and impurities were prepared protected from light and used within two hours of preparation to prevent degradation.

Sample Solutions

A mass of tablet corresponding to 12.5 mg lercanidipine hydrochloride was dissolved in mobile phase, in a 25-mL volumetric flask, by immersion in an ultrasonic bath for 30 min. The solution was then centrifuged at 4,000 rpm for 5 min.

Solutions were prepared protected from light and used within two hours of preparation to prevent degradation.

Chromatographic Conditions

Chromatography was performed with a Hewlett–Packard (HP) 1100 system (Agilent, Technologies, Germany) comprising an HP 1100 autosampler, an HP 1100 pump, an HP 1100 UV–visible detector, and an HP ChemStation integrator. Compounds were separated on a 20 × 4.6 mm, 3.5-µm particle size, XTerra C₁₈ column at 20 °C. Samples were introduced by means of the autosampler.

Separation and simultaneous analysis of lercanidipine hydrochloride and impurities 1, 3, and B were performed with mobile phase which consisted of 350 mL acetonitrile and 650 mL water; the water contained 1.5% TEA and the pH was adjusted to 3.0 with orthophosphoric acid. Before use the mobile phase

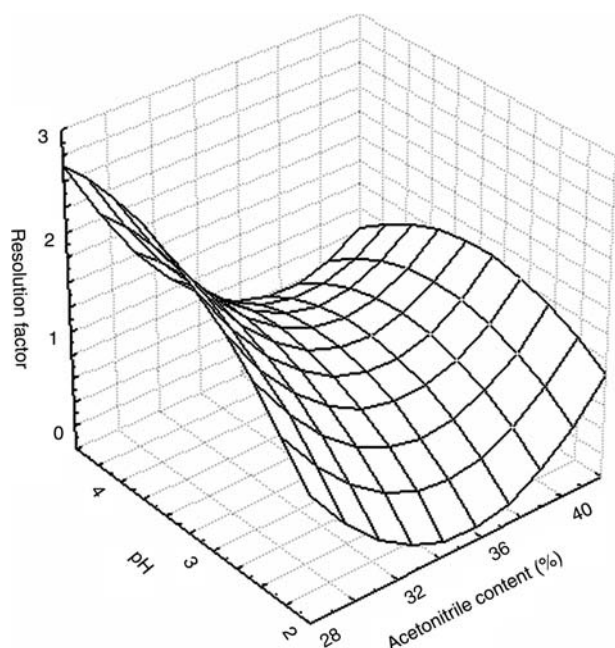


Fig. 3. Three-dimensional graph showing the dependence of the resolution of impurities B and 1 on mobile phase ACN content and on pH

was filtered through a 0.2- μm Millipore filter. The flow rate was 1.0 mL min^{-1} , the column temperature $20 \text{ }^\circ\text{C}$, and the injection volume $20 \mu\text{L}$; UV detection was at 240 nm .

Results were evaluated with DryLab 2000 Plus chromatography optimization software version 3.5.00 and Statistica 7 software.

Results and Discussion

Method Development

In RP-HPLC, retention is significantly affected by the polarity and ionic strength of the mobile phase and the polarity of the stationary phase. The retention of substances is also related to their physicochemical properties, for example hydrophobicity ($\log P$), and the possibility of ion-pairing, ionization, etc. The basic character of lercanidipine free base and impurity 3 must be taken into account, because it will strongly affect their chromatographic behavior. Also the similarity of their structures may be of utmost importance for setting the optimum separation conditions. On the other hand, impurities B and 1 have free

carboxyl groups and differ in polarity from lercanidipine and impurity 3. Preliminary experiments conducted on non-polar columns showed that mobile phase pH should be from 2.0 to 4.0 and addition of TEA was obligatory to achieve acceptable peak symmetry for lercanidipine hydrochloride and impurity 3. Column temperature had no effect on the separation.

In all subsequent experiments 1.5% TEA was added to the aqueous component of the mobile phase and the pH of this component was adjusted to 2.0, 3.0, or 4.0 with orthophosphoric acid. It was found the acetonitrile-to-water ratio could be varied from 30:70 to 40:60 (v/v). The best initial results, for example the best peak symmetry and the shortest run, were achieved by use of an XTerra column, so this was used in subsequent experiments.

To find the best conditions for separation of lercanidipine hydrochloride and its impurities 3^k factorial design and DryLab software were used. Acetonitrile content and pH of the aqueous component of the mobile phase were factors extracted from preliminary experiments to be closely studied in method optimization. In software optimization the

factors chosen were changed sequentially; in optimization by factorial design, however, factors were changed simultaneously.

The inputs required by the software to construct resolution maps were compound retention times and peak areas, column properties, instrumental factors, and flow rate. In experiments with DryLab the factor of interest was changed while other factors were kept constant. First, acetonitrile was investigated at two levels (30 and 40%), then mobile phase pH at three levels (2.0, 3.0, and 4.0). The resolution maps obtained (Fig. 2a, b) are the best way of visualizing the effect of the causal factors on the resolution of a critical pair of substances (in this work impurities B and 1).

Because DryLab requires a few experiments for setting of the optimum conditions, the authors recognized the need to perform further analysis to confirm data obtained from the software. The same two factors as in DryLab, at three levels, were analyzed using a 3^2 design, and nine experiments were performed. To compare DryLab and experimental design, the resolution of the critical pair (impurities B and 1) was chosen as output.

The best way to present and, at the same time, predict system behavior from the range of factors investigated is by creation of a 3D graph. The three-dimensional graph of resolution of the critical pair is presented in Fig. 3.

According to the resolution maps and 3D graphs the most appropriate chromatographic separation will be achieved with a 35:65 (v/v) mixture of acetonitrile and aqueous phase (1.5% (v/v) TEA, adjusted to pH 3 with orthophosphoric acid). Both methods of optimization give similar conditions as optimum. The advantage of DryLab is that a small number of experiments is sufficient for identification of the optimum conditions. Data obtained from experimental design offer a more precise description of the system, however. Three-dimensional graphs facilitate prediction of retention times in the domain of the factors investigated. The more descriptive representation of the system acquired by experimental design enables survey and adaptation of differ-

ent conditions. In this case, results from the methods if optimization were harmonized.

Method Validation

After establishing the optimum conditions for the separation, the selectivity, linearity, precision, robustness, and limits of quantification and detection were determined, i.e. fundamental attributes of the method were examined.

Representative chromatograms obtained from a laboratory mixture and the corresponding tablets, under the optimum conditions, are shown in Fig. 4a, b.

The assay was selective, no significant interfering peaks were observed at the retention times of lercanidipine hydrochloride or any of the impurities. Excipients from the tablets were eluted at different times and did not interfere with the compounds analyzed.

Linear relationships between response and amount chromatographed were obtained for lercanidipine hydrochloride, and impurities 1, 3, and B over the concentration ranges investigated (Table 1). Because the correlation coefficients (r) for the calibration plots for lercanidipine hydrochloride and its three impurities were >0.9991 it can be concluded the calibration plots are within acceptance criteria for linearity.

Method accuracy was expressed as percentage recovery. For lercanidipine hydrochloride in samples recovery ranged from 99.2 to 100.9%. Recovery of the impurities varied from 99.1 to 101.8% for impurity B, from 97.1 to 99.2% for impurity 1, and from 99.4 to 101.5% for impurity 3.

During study of the precision of the assay for lercanidipine, RSD was $\leq 0.9\%$; for impurities B, 1, and 3 the RSD of peak area was 1.4, 2.0, and $\leq 2.3\%$, respectively. These RSD confirmed the high precision of the method.

The large effect of acetonitrile on the retention of all the compounds is obvious and expected, so the amount of organic solvent must be strictly controlled. The separation is robust if the acetonitrile content is from 34 to 36% (v/v). Small changes of mobile phase pH, on

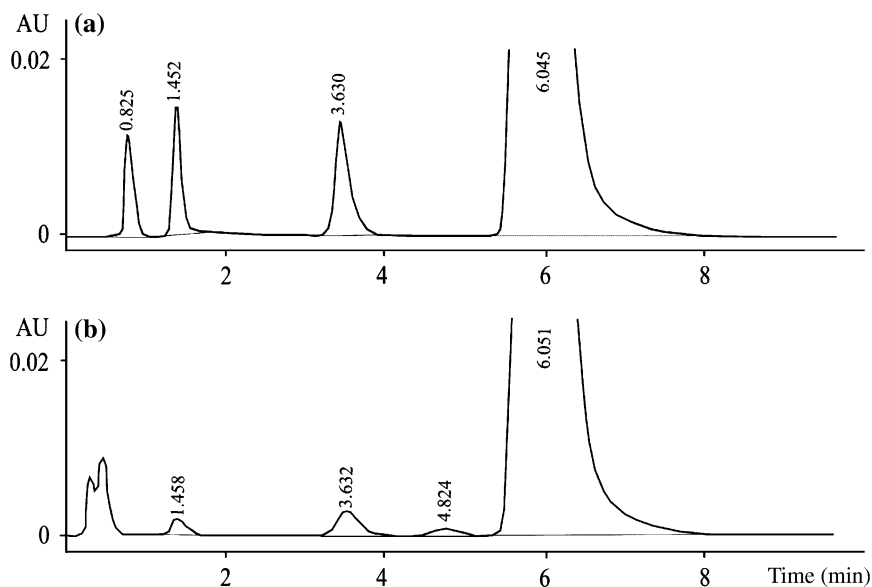


Fig. 4. Chromatograms obtained from lercanidipine hydrochloride ($t_R = 6.045$ min) and impurities B ($t_R = 0.825$ min), 1 ($t_R = 1.452$ min), and 3 ($t_R = 3.630$ min) (a) in a laboratory mixture and (b) from tablets. The mobile phase was 35:65 (v/v) acetonitrile–water (1.5% TEA, pH 3.0 adjusted with orthophosphoric acid), the flow rate 1.0 mL min^{-1} , UV detection was 240 nm, and the temperature was 20°C

the other hand, did not have a significant effect on the separation of API and its impurities.

For quantitative analysis it was important to define the limits of detection (LOD) and quantification (LOQ) (Table 1). LOD and LOQ were defined as the amounts for which the signal-to-noise ratios were 3:1 and 10:1, respectively, and further confirmed by dilution of the secondary stock solution until the peak area obtained was three (for LOD) and ten (for LOQ) times the standard deviation for six determinations.

Application of the Optimized and Validated Method for Pharmaceutical Dosage Form Analysis

After evaluation of the method it was used for determination of the amounts of lercanidipine hydrochloride, and impurities 1, 3, and B in commercially available Carmen tablets. The lercanidipine hydrochloride content was 100.6% (RSD 0.34%) and the levels of the impurities met stipulated requirements well, i.e. impurity 1, 0.018% (RSD

3.8%), impurity 3, 0.17% (RSD 0.2%), and impurity B below the LOD. The results obtained by quantitative analysis are in a good agreement with the declared content.

Conclusion

In method development, method optimization is the first step which gives directions for further investigations, for method validation, etc. If all aspects of the method are analyzed in the first stage, unpredictable results cannot occur. In this paper DryLab and 3^2 experimental design were used to achieve the most appropriate separation of lercanidipine and its three degradation product (B, 1, 3). The advantage of DryLab is that only four experiments are sufficient for isolation of the optimum conditions; experimental design offers more descriptive representation, however. Because results from both optimization methods were harmonized the approach proposed is versatile. The actual method used will depend mainly on the purpose of the investigation.

Table 1. Calibration and validation data

	Lercanidipine hydrochloride	Impurity B	Impurity 1	Impurity 3
Concentration range	0.07–1.0 mg mL ⁻¹	0.21–3.0 µg mL ⁻¹	0.21–3.0 µg mL ⁻¹	0.21–3.0 µg mL ⁻¹
$y = ax + b$	$y = 44043.56x + 31.39$	$y = 28.6x + 0.53$	$y = 125.95x + 4.87$	$y = 24.58x + 0.62$
R	0.9999	0.9991	0.9998	0.9998
S_b	66.99	1.22	2.71	0.44
LOQ (µg mL ⁻¹)	0.05	0.03	0.03	0.06
LOD (µg mL ⁻¹)	0.02	0.01	0.01	0.02

r correlation coefficient, S_b standard deviation of the intercept

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