ORIGINAL ARTICLE

Novel and validated titrimetric method for determination of selected angiotensin-II-receptor antagonists in pharmaceutical preparations and its comparison with UV spectrophotometric determination

Shrikant H. Patil\textsuperscript{a,\*}, Minakshi V. Janjale\textsuperscript{b}

\textsuperscript{a}Department of Quality Assurance Techniques, N.D.M.V.P Samaj’s College of Pharmacy, Gangapur Road, Nasik 422002, Maharashtra, India
\textsuperscript{b}Department of Pharmaceutics, S.S.J.I.P.E.R, Jamner 424206, Maharashtra, India

Received 6 December 2011; accepted 30 March 2012
Available online 9 April 2012

KEYWORDS
Angiotensin-II-receptor antagonists; Titrimetric assay; UV spectrophotometry; Validation

Abstract A novel and simple titrimetric method for determination of commonly used angiotensin-II-receptor antagonists (ARA-IIs) is developed and validated. The direct acid base titration of four ARA-IIs, namely eprosartan mesylate, irbesartan, telmisartan and valsartan, was carried out in the mixture of ethanol:water (1:1) as solvent using standardized sodium hydroxide aqueous solution as titrant, either visually using phenolphthalein as an indicator or potentiometrically using combined pH electrode. The method was found to be accurate and precise, having relative standard deviation of less than 2% for all ARA-IIs studied. Also, it was shown that the method could be successfully applied to the assay of commercial pharmaceuticals containing the above-mentioned ARA-IIs. The validity of the method was tested by the recovery studies of standard addition to pharmaceuticals and the results were found to be satisfactory. Results obtained by this method were found to be in good agreement with those obtained by UV spectrophotometric method. For UV spectrophotometric analysis ethanol was used as a solvent and wavelength of 233 nm, 246 nm, 296 nm, and 250 nm was selected for determination of eprosartan mesylate, irbesartan, telmisartan, and valsartan respectively. The proposed titrimetric method is simple, rapid, convenient and sufficiently precise for quality control purposes.

© 2012 Xi’an Jiaotong University. Production and hosting by Elsevier B.V. Open access under CC BY-NC-ND license.

1. Introduction

Many of the active components of pharmaceutical preparations are of organic origin and contain acidic or basic groups. Such compounds can be successfully determined in their pharmaceutical preparations using titrimetric methods. The purpose of this work was to develop a simple, accurate, reproducible and rapid titrimetric method for the determination of commonly used angiotensin-II-receptor antagonists.
(ARA-IIs) such as eprosartan mesylate (I), irbesartan (II), telmisartan (III) and valsartan (IV), and applying it to the pharmaceutical dosage forms. These compounds contain either carboxylic acid group or tetrazole ring or both which act as an acidic centre and form the basis for acid-base reactions during titration. The structural formulae of these ARA-IIs are given in Fig. 1.

These ARA-IIs are safe and effective agents in the treatment of hypertension and heart failure, either alone or in conjunction with diuretics. They have been proposed as alternatives to the more traditional angiotensin converting enzyme (ACE) inhibitors because they selectively block the angiotensin type 1 (AT1) receptor, which is responsible for vasoconstriction, and salt and water retention. The angiotensin type 2 (AT2) receptor, which is thought to have cardioprotective effects and inhibitory effects on growth, is left unaffected [1–6].

Several methods that are reported for ARA-IIs compounds estimation include enzyme-linked immunosorbent assays (ELISAs) for the determination of telmisartan in human blood plasma [7], spectrophotometric for the determination of valsartan in human urine [8], colorimetric method [9], and UV-derivative spectrophotometric [10] for the determination of ARA-II in bulk and in tablets. Tatar and Saglik [11] compared UV- and second derivative-spectrophotometric and high-performance liquid chromatographic methods for the determination of valsartan in pharmaceutical formulation. Also, capillary electrophoresis (CE), capillary electrochromatography (CEC), micellar electrokinetic capillary chromatography (MEKC) and capillary zone electrophoresis (CZE) methods have also been reported [12–16]. High-performance liquid chromatography has been the major technique used in the determination of these compounds in different matrices with UV [17–24], fluorimetric [25–27] or mass spectrometry (MS) detections [28–30]. Validated methods which allow the determination of a single drug [31–39] or combination of ARA-IIs with hydrochlorothiazide or some of their metabolites [40–43] in urine, plasma and in pharmaceutical formulations [44] have also been published.

Although chromatographic techniques have been suggested for the determination of ARA-IIs, it requires high skilful operator and expensive instrument. In addition, most of the described procedures require expensive instrumental setup. So, there is a need to develop a simple, reliable, rapid and economical method for the determination of ARA-IIs in pharmaceuticals.

No titrimetric method for determination of ARA-IIs has been found in literature. In this paper, the validated titrimetric method is described for the determination of ARA-IIs in pharmaceuticals. The method is based on the titration of the drug solution in neutral ethanol:water mixture (1:1) with aqueous NaOH to a phenolphthalein end point or potentiometric equivalence point. In this paper the proposed titrimetric method is a very simple technique and adoptable for routine analysis to determine the content of ARA-IIs at milligram level in the quality control laboratories.

Because of unavailability of pharmacopial method for some of these ARA-IIs, UV spectrophotometric method has been developed for statistical comparison with results obtained by proposed titrimetric method. A comparison of results obtained by the proposed titrimetric method and those obtained by UV method shows good statistical correlation.

2. Materials and methods

2.1. Apparatus

A Jenway 3020 digital pH meter equipped with a combined pH-electrode was used throughout the study. All titrations were carried out manually. A shimadzu UV–visible recording spectrophotometer (model UV2501 PC) with 1 cm matched quartz cells was used for spectrophotometric analysis.

2.2. Reagents and materials

Eprosartan mesylate, valsartan, and telmisartan were obtained from Glenmark Pharmaceutical Ltd. Sinnar, Nasik, India; and irbesartan was obtained from Cadila Healthcare Ltd., Ahmedabad, India. These ARA-IIs were chemically pure laboratory working standards having purities of 99.8%, 99.4%, 99.6% and 99.3%. Sodium hydroxide, ethanol, potassium hydrogen phthalate, and phenolphthalein powder were obtained from Merck, India and S.D’s Lab Chem & Industries, Bombay. Teveten (eprosartan mesylate), Karvea (irbesartan), Telsartan (telmisartan) and Diovan (valsartan) tablets were obtained from a local pharmacy. All chemicals were of analytical reagent grade unless otherwise stated, and doubly distilled deionised water was used throughout.

Sodium hydroxide (0.01 M): Accurately 0.2 g of the pure NaOH (Merck, India) was dissolved in doubly distilled water. The solution was made up to 500 mL with the same water and standardized [45].

Phenolphthalein indicator (0.5%): It was prepared by dissolving 500 mg of the pure phenolphthalein powder (S.D’s Lab Chem & Industries, Bombay) in 50 mL alcohol and diluting to 100 mL with doubly distilled water.

2.3. Procedures

2.3.1. Potentiometric titration

Accurately weighed quantities (2.0–10.0 mg) of four ARA-IIs, namely eprosartan mesylate, irbesartan, telmisartan and

![Figure 1](image-url)
valsartan, were dissolved separately in 20 mL mixture (1:1) of ethanol and water, depending upon their molar weights. Ethanol should be previously neutralized to phenolphthalein solution. All the assay solutions were prepared prior to titrations directly in a titration cell, and titrated with standardized sodium hydroxide aqueous solution using potentiometric titration with a combined platinum ring electrode. Near the equivalence point, titrant was added in 0.05 mL increments. After each addition of titrant, the solution was stirred magnetically for 30 s and the steady potential was noted. The addition of titrant was continued until no significant change in potential on further addition of titrant. The equivalence point was determined by applying the graphical method. The amount of the drug in the measured aliquot was calculated from:

\[
\text{Amount (mg)} = VMwR/n
\]

where \( V \) is the volume of NaOH required, mL; \( Mw \) is the relative molecular mass of the drug; \( R \) is the molarity of NaOH and \( n \) is the number of moles of NaOH reacting with each mole of the drug.

2.3.2. **Visual titration**

Accurately weighed quantities (2.0–10.0 mg) of four ARA-IIs, namely eprosartan mesylate, irbesartan, telmisartan and valsartan, were dissolved separately in a mixture of 10 mL of water and 10 mL of neutral ethanol depending upon their molar weights. All the assay solutions were titrated with standardized sodium hydroxide aqueous solution using 2–4 drops of 0.5% phenolphthalein indicator to a pink colour end point. The amount of the drug in the measured aliquot was calculated as described under potentiometric titration.

2.3.3. **Titrimetric determination of ARA-IIs from pharmaceutical preparations**

Twenty tablets were weighed, and their average weights were calculated. All the tablets were finely powdered and the required amounts of these powders were dissolved in a mixture of 10 mL of water and 10 mL of ethanol. The mixture was sonicated for 5 min and filtered using Whatmann No 42 filter paper. A suitable aliquot was next subjected to analysis by potentiometry and visual titration method as described earlier.

The titrations were repeated for different amounts of each ARA-II and pharmaceutical preparation.

2.3.4. **UV-spectrophotometric method**

For obtaining calibration curve for UV-method, a series of solutions were prepared for each ARA-II within their Beer–Lambert’s range of concentration as shown in Table 1, by diluting the respective stock ARA-II solution (0.1 mg/mL in ethanol) with ethanol in volumetric flasks (10 mL). The absorbance of each solution was determined at respective lambda max of the drug as shown in Table 1 against ethanol as blank. A calibration curve was prepared by plotting absorbance versus concentration for each ARA-II. Absorption spectra of ARA-IIs are given in Fig. 2.

### Table 1  Summary of optical characteristics and validation parameters of ARA-IIs.

| Parameters                  | Eprosartan mesylate | Irbesartan | Telmisartan | Valsartan |
|-----------------------------|---------------------|------------|-------------|-----------|
| Lambda max (nm)             | 233                 | 246        | 296         | 250       |
| Beer’s law limit (range) (µg/mL) | 6–36               | 2–36       | 4–30        | 2–20      |
| Correlation coefficient (±S.D.) | 0.999 ± 0.690      | 0.999 ± 0.450 | 0.999 ± 0.390 | 0.999 ± 0.560 |
| Regression equation         | \( Y=0.056x+0.023 \) | \( Y=0.036x+0.023 \) | \( Y=0.056x+0.023 \) | \( Y=0.0328x+0.0206 \) |
| LOD (µg/mL)                 | 0.4142              | 0.5119     | 0.2579      | 0.1337    |
| LOQ (µg/mL)                 | 1.2552              | 1.5495     | 0.7805      | 0.4052    |

### 2.3.5. UV spectroscopic determination of ARA-IIs from pharmaceutical preparations

Twenty tablets were weighed, and their average weights were calculated. All the tablets were finely powdered and the required amounts of these powders were dissolved in 20 mL of ethanol. The mixture was sonicated for 5 min and filtered using Whatmann No 42 filter paper. After suitable dilution, absorbance was recorded against the blank at respective lambda max of drug as shown in Table 1.

### 2.4. Method validation

Both potentiometric and UV spectroscopic methods were validated in compliance with ICH guidelines. The following parameters were validated.

#### 2.4.1. Precision

The precision of the potentiometric and UV spectroscopic methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different amounts of ARA-IIs within the range of study in each method were analyzed in five replicates during the same day (intra-day precision) and five consecutive days (inter-day precision).

#### 2.4.2. Recovery studies

Accuracy and the reliability of both methods were ascertained by performing recovery experiments. To a fixed amount of drug in formulation (pre-analyzed); pure drug at three different levels corresponding to its 80%, 100% and 120% was added (standard addition method), and the total was found by the proposed methods. Each test was repeated three times and the results obtained were compared with expected results.

#### 2.4.3. Ruggedness

Ruggedness of both methods was done at three different concentration levels of each ARA-II within the range of study in each method.

Ruggedness of potentiometric method was expressed as the RSD of the same procedure applied by three different analysts as well as using three different burettes.

The ruggedness of the UV spectroscopic method was determined by carrying out the experiment on three different instruments and by three different analysts.
2.4.4. Linearity

2.4.4.1. Potentiometric method. For the establishment of method linearity, five different weights of ARA-IIs test samples corresponding to 20%, 40%, 60%, 80% and 100% of the about weight 20 mg were taken and analyzed potentiometrically. Calibration curve was drawn by plotting test sample weight on X axis and titre values on Y axis. The values of correlation coefficient, slope and intercept were determined.

2.4.4.2. UV spectroscopic method. Appropriate dilutions of standard stock solutions of each ARA-II were analyzed as per the developed methods. Beer–Lambert’s concentration range and linearity data were determined.

2.4.5. LOD and LOQ
For UV method, limit of detection (LOD) and limit of quantification (LOQ) of each ARA-II were calculated as $3.3 \delta / S$ and $10 \delta / S$, respectively as per ICH guidelines, where $\delta$ is the standard deviation of the response and $S$ is the slope of the calibration plot. The LOD is the smallest concentration of the analyte that gives a measurable response. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified.

3. Results and discussion

3.1. Titrmetric measurements

3.1.1. Potentiometric determination of standard active components
ARA-IIs were titrated direct potentiometrically in a mixture of ethanol and water (1:1) using standardized sodium hydroxide aqueous solution as a titrant. The titration curve of ARA-IIs showed one well-defined S-shaped stoichiometric end-point (Fig. 3). The determination of the end points from the potentiometric data was carried out using the Gran’s method [46].

Table 2 gives detail about acidic centres present in ARA-IIs which corresponds to the number of equivalent of bases required for neutralization to have the end point. For example the end point of telmisartan corresponded to one equivalent of base and was related to the neutralization of one –COOH group.

The percentage of each ARA-II (chemically pure laboratory working standard) was calculated from the potentiometric titration data. Five successive determinations were carried out for each ARA-II. The results are tabulated in Table 3. As seen from the data in Table 3, the mean values obtained by the proposed method are in good agreement with the nominal value given for each ARA-II and furthermore the relative standard deviations are less than 1%. This indicates that the accuracy and the precision of this method are satisfactory.
3.1.2. Visual titrimetric determination of standard active components

The percentage of each ARA-II (chemically pure laboratory working standard) was calculated from the visual titration data. Five successive determinations were carried out for each ARA-II. As seen from the data in Table 3, results obtained by this method were found to be in good agreement with those obtained by potentiometric method.

3.1.3. Titrimetric determination of ARA-IIs in dosage forms

In order to evaluate the applicability of the above-mentioned titrimetric methods to pharmaceutical preparations, ARA-IIs were determined in their pharmaceutical preparations respectively, under the same conditions as employed for the pure anti-inflammatory agents. The fact that the mV values before the end-points in the titration curves of pure anti-inflammatory agents and their corresponding pharmaceuticals are almost identical provides evidence that the titration curves are not due to other excipients that might be present in the pharmaceutical preparations and excipients do not affect the titration curves. The excipients in the above-mentioned pharmaceutical preparation do not include acidic substances.

Table 3 summarizes the results obtained for each anti-inflammatory agent in the pharmaceutical preparations, expressed as percentages of the nominal contents. The results are in good agreement with the nominal contents and the RSD values are less than 1%. Thus, the reproducibility and accuracy are very satisfactory for the analysis of pharmaceutical preparations as well as bulk drugs.

3.2. UV-spectrophotometric measurements

Lambda max, Beer’s law limit (concentration range), correlation coefficient (r) and regression equation obtained by UV-spectrophotometric method for each ARA-II are given in Table 1. The proposed UV-spectrophotometric method was also successfully applied for the determination of ARA-IIs in some pharmaceutical preparations (Table 4). The results are in good agreement with the nominal contents and the RSD values are less than 1%. Thus, the reproducibility and accuracy are very satisfactory for the analysis of pharmaceutical preparations as well as bulk drugs.

3.3. Validation

3.3.1. Recovery studies

The recovery studies of standard additions to commercial pharmaceuticals were carried out in order to determine accuracy and selectivity of the method. In these titrations, as the amount of pure standard added to commercial pharmaceuticals increases, the volume of titrant used increases linearly. The results related to these studies are presented in Table 5. It can be seen from this Table that the mean recoveries and RSD values are good evidence of the accuracy of the method.

3.3.2. Precision

The RSD values of intra-day and inter-day precision of the potentiometric and UV spectroscopic methods for all ARA-IIs showed that the precision of both methods was good (Table 6).

3.3.3. Ruggedness

The RSD values of inter analyst as well as inter instrument analysis were less than 2% for potentiometric method as well as UV spectrometric method. This proves good ruggedness of the method (Table 7).

3.3.4. LOD and LOQ

For UV method, LOD and LOQ of each ARA-II are presented in Table 1.
3.3.5. Comparison between developed methods
ARA-IIs were determined in pharmaceutical preparations by the developed methods and the results obtained are presented in Table 4 where excellent agreement between the three procedures can be observed. The statistical comparison of the results shows that, there is no significant difference between results of UV-spectrophotometric and potentiometric methods ($t=1.49$, $F=1.16$); UV-spectrophotometric and visual titrimetric methods ($t=1.18$, $F=1.33$) since the calculated $t$- and $F$-tests did not exceed the theoretical values ($n=5$, $p=0.05$, $t=2.23$, $F=5.05$) at the 95% confidence level (Table 8).

4. Conclusion
Statistical tests indicate that the proposed titrimetric and UV methods appear to be equally suitable for routine determination...
of ARA-IIs in pharmaceutical formulation. As a result of this work, ARA-IIs can now be determined titrimetrically by the proposed method. This aqueous titrimetric assay was successfully applied to the determination of pure authentic samples and some of their pharmaceutical preparations. In the proposed method, the titrations of all ARA-IIs have shown rather well shaped endpoints with high potential jumps. In conclusion, the proposed titrimetric method offers a simple system and with the short analytical time, coupled with good reproducibility, accuracy, ruggedness and cost-effectiveness.

Acknowledgments

The authors are thankful to NDMVP college of pharmacy, Nashik for helping in this research work.

References

[1] B. Pitt, M.A. Konstam, Overview of angiotensin II-receptor antagonists, Am J Cardiol 82 (1998) 47S–48S.
[2] R. Willenheimer, B. Dahlof, E. Rydberg, et al., AT1-receptor blockers in hypertension and heart failure: clinical experience and future directions, Eur Heart J 20 (1999) 997–998.
[3] I.C. Johnston, M. Naitoh, L.M. Burrell, Rationale and pharmacology of angiotensin II receptor antagonists: current status and future issues, J Hypertens Suppl. 15 (1997) S3–S4.
[4] T. Unger, Significance of angiotensin type I receptor blockade: why are angiotensin II receptor blockers different, Am J Cardiol 84 (1999) 9S–10S.
[5] B. Porta Oltra, C. Borrás Almenar, N.V. Jiménez Torres, Therapeutic interchange standardization for angiotensin II receptor antagonists in the treatment of hypertension in the hospital setting, Farm Hosp 29 (2005) 104–112.
[6] B. Schmidt, B. Schieffer, Angiotensin II AT1 receptor antagonists: clinical implications of active metabolites, J Med Chem 46 (2003) 2261–2270.
[7] Ch. Hempen, L. Gisèle-Schwarz, U. Kunz, et al., Determination of telmisartan in human blood plasma: Part I: immunoassay development, Anal Chim Acta 560 (2006) 35–40.
[8] E. Cagigal, L. González, R.M. Alonso, et al., Experimental design methodologies to optimise the spectrofluorimetric determination of Losartan and Valsartan in human urine, Talanta 54 (2001) 1121–1133.
[9] A.H. Prabhakar, R. Giridhar, A rapid colorimetric method for the determination of Losartan potassium in bulk and in synthetic mixture for solid dosage form, J Pharm Biomed. Anal 27 (2002) 861–866.
[10] O.C. Lastra, I.G. Lemus, H.J. Sánchez, et al., Development and validation of an UV derivative spectrophotometric determination of Losartan potassium in tablets, J Pharm Biomed. Anal. 33 (2003) 175–180.
[11] S. Tatar, S. Saglik, Comparison of UV- and second derivative-spectrophotometric and LC methods for the determination of valsartan in pharmaceutical formulation, J Pharm Biomed. Anal. 30 (2002) 371–375.
[12] M.G. Quaglia, E. Donati, G. Carlucci, et al., Determination of losartan and hydrochlorothiazide in tablets by CE and CEC, J Pharm. Biomed. Anal. 29 (2002) 981–987.
[13] M. Zhang, F. Wei, Y.F. Zhang, et al., Novel polymer monolith microextraction using a poly(methacrylic acid-ethylene glycol dimethacrylate) monolith and its application to simultaneous analysis of several angiotensin II receptor antagonists in human urine by capillary zone electrophoresis, J Chromatogr A 1102 (2006) 294–301.
[14] S. Hillaert, T.R. De Beer, J.O. De Beer, et al., Optimization and validation of a micellar electrokinetic chromatographic method for the analysis of several angiotensin-II-receptor antagonists, J Chromatogr A 984 (2003) 135–146.
[15] R.C. Williams, M.S. Alasandro, V.L. Fasone, et al., Comparison of liquid chromatography, capillary electrophoresis and supercritical fluid chromatography in the determination of Losartan Potassium drug substance in Cozaar tablets, J Pharm. Biomed. Anal. 14 (1996) 1539–1546.
[16] S. Hillaert, W. Van den Bossche, Simultaneous determination of hydrochlorothiazide and several angiotensin-II-receptor antagonists by capillary electrophoresis, J. Pharm. Biomed. Anal. 31 (2003) 329–339.
[17] J. Nie, M. Zhang, Y. Fan, et al., Biocompatible in-tube solid-phase microextraction coupled to HPLC for the determination of angiotensin II receptor antagonists in human plasma and urine, J Chromatogr B 787 (2005) 62–69.
[18] D.E. Lundberg Jr., C.R. Person, S. Knox, et al., Determination of SK and F 108566 (Teveten(R)) in human plasma by reversed-phase high-performance liquid chromatography, J Chromatogr. B 707 (1999) 328–329.
[19] S.Y. Chang, D.B. Whigan, N.N. Vachharajani, et al., High-performance liquid chromatographic assay for the quantitation of irbesartan (SR 47346/BMS-186295) in human plasma and urine, J Chromatogr. B 768 (1999) 77–78.
[20] A. Soldner, H. Spahn-Langguth, E. Mutschler, HPLC assays to simultaneously determine the angiotensin-AT1 antagonist losartan as well as its main and active metabolite EXP 3174 in biological material of humans and rats, J Pharm. Biomed. Anal. 16 (1998) 863–864.
[21] D. Farthing, M. Sica, I. Fakhry, et al., Simple high-performance liquid chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate, J Chromatogr. B 704 (1997) 374–375.
[22] H. Lee, H.O. Sim, H.S. Lee, Simultaneous determination of losartan and active metabolite EXP3174 in rat plasma by HPLC with column switching, Chromatographia 42 (1996) 39–40.
[23] C.I. Furtek, M.W. Lo, Simultaneous determination of a novel angiotensin II receptor blocking agent, losartan, and its metabolite in human plasma and urine by high-performance liquid chromatography, J Chromatogr. 111 (1992) 295–296.
[24] L. González, J.A. López, R.M. Alonso, et al., Fast screening method for the determination of angiotensin II receptor antagonists in human plasma by high-performance liquid chromatography with fluorimetric detection, J. Chromatogr. A 949 (2002) 49–60.
[26] H. Stenhoff, P.O. Lagerstrom, C. Andersen, Determination of candesartan cilexetil, candesartan and a metabolite in human plasma and urine by liquid chromatography and fluorometric detection, J. Chromatogr. B 731 (1999) 411–412.

[27] M.A. Ritter, C.I. Furtek, M.W. Lo, An improved method for the simultaneous determination of losartan and its major metabolite, EXP3174, in human plasma and urine by high-performance liquid chromatography with fluorescence detection, J. Pharm. Biomed. Anal. 15 (1997) 1021–1022.

[28] L. Kristoffersen, E. Leere Oiestad, M. Stokke Opdal, et al., Simultaneous determination of 6 beta-blockers, 3 calcium-channel antagonists, 4 angiotensin-II antagonists and 1 antiarrhythmic drug in post-mortem whole blood by automated solid phase extraction and liquid chromatography mass spectrometry, Method development and robustness testing by experimental design, J. Chromatogr. B 850 (2007) 147–160.

[29] T. Iwasa, T. Takano, K. Hara, et al., Method for the simultaneous determination of losartan and its major metabolite, EXP-3174, in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry, J. Chromatogr. B 734 (1999) 325–326.

[30] Z.X. Zhao, Q.X Wang, E.W. Tsai, et al., Identification of losartan degradates in stressed tablets by LC-MS and LC-MS/MS, J. Pharm. Biomed. Anal. 20 (1999) 129–130.

[31] N. Ferreiros, G. Iriarte, R.M. Alonso, et al., Development of a solid phase extraction procedure for HPLC-DAD determination of several angiotensin II receptor antagonists in human urine using mixture design, Talanta 73 (2007) 748–756.

[32] N. Koseki, H. Kawashita, H. Hara, et al., Development and validation of a method for quantitative determination of valsartan in human plasma by liquid chromatography–tandem mass spectrometry, J. Pharm. Biomed. Anal. 43 (2007) 1769–1774.

[33] N. Torrealday, L. González, R.M. Alonso, et al., Experimental design approach for the optimisation of a HPLC–fluorimetric method for the quantitation of the angiotensin II receptor antagonist telmisartan in urine, J. Pharm. Biomed. Anal. 32 (2003) 847–857.

[34] A.K. Shakya, Y.M. Al-Hiari, O.M.O. Alhamami, Liquid chromatographic determination of irbesartan in human plasma, J. Chromatogr. B 848 (2007) 245–250.

[35] P. Li, Y. Wang, Y. Wang, et al., Determination of telmisartan in human plasma by liquid chromatography–tandem mass spectrometry, J. Chromatogr. B 828 (2005) 126–129.

[36] J. Macek, J. Klima, P. Ptácek, Rapid determination of valsartan in human plasma by protein precipitation and high-performance liquid chromatography, J. Chromatogr. B 832 (2006) 169–172.

[37] N. Ferreiros, G. Iriarte, R.M. Alonso, et al., Validation of a solid phase extraction–high performance liquid chromatographic method for the determination of eprosartan in human plasma, J. Chromatogr. A 1119 (2006) 309–314.

[38] D. Liu, P. Hu, N. Matsushima, et al., Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. B 856 (2007) 190–197.

[39] N. Daneshtalab, R.Z. Lewanczuk, F. Jamali, High-performance liquid chromatographic analysis of angiotensin II receptor antagonist valsartan using a liquid extraction method, J. Chromatogr. B 766 (2002) 345–349.

[40] N. Erk, Simultaneous determination of irbesartan and hydrochlorothiazide in human plasma by liquid chromatography, J. Chromatogr. B 784 (2003) 195–201.

[41] H. Li, Y Wang, Y. Jiang, et al., A liquid chromatography/tandem mass spectrometry method for the simultaneous quantification of valsartan and hydrochlorothiazide in human plasma, J. Chromatogr. B 852 (2007) 436–442.

[42] M. Polinko, K. Rifle, H. Song, et al., Simultaneous determination of losartan and EXP3174 in human plasma and urine utilizing liquid chromatography/tandem mass spectrometry, J. Pharm. Biomed. Anal. 33 (2003) 73–84.

[43] L. González, R.M. Alonso, R.M. Jiménez, A high-performance liquid chromatographic method for screening angiotensin II receptor antagonists in human urine, Chromatographia 52 (2000) 735–740.

[44] D.L. Hertzog, J. Finnegan McCafferty, X. Fang, et al., Development and validation of a stability-indicating HPLC method for the simultaneous determination of Losartan potassium, hydrochlorothiazide, and their degradation products, J. Pharm. Biomed. Anal. 30 (2002) 747–760.

[45] A.I. Vogel, Quantitative Inorganic Analysis Including Elementary Instrumental Analysis, 3rd ed., The English Language Book Society and Longman, London, 1962, pp. 242–243.

[46] E.P. Serjeant, Potentiometry and Potentiometric Titrations, Wiley, New York, 1984, pp. 98–99.