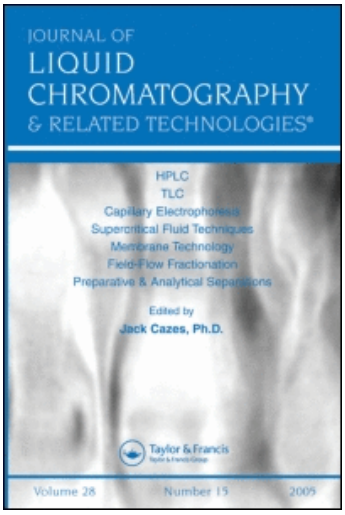

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DEVELOPMENT OF LLE AND SPE PROCEDURES AND ITS APPLICATIONS FOR DETERMINATION OF OLMESARTAN IN HUMAN PLASMA USING RP-HPLC AND HPTLC

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Online publication date: 08 February 2010

To cite this Article Tambe, Santosh R. , Shinde, Rupali H. , Gupta, Lalit R. , Pareek, Vikas and Bhalerao, Santosh B.(2010) 'DEVELOPMENT OF LLE AND SPE PROCEDURES AND ITS APPLICATIONS FOR DETERMINATION OF OLMESARTAN IN HUMAN PLASMA USING RP-HPLC AND HPTLC', Journal of Liquid Chromatography & Related Technologies, 33: 4, 423 — 430
To link to this Article: DOI: 10.1080/10826070903571358
URL: http://dx.doi.org/10.1080/10826070903571358

Journal of Liquid Chromatography & Related Technologies, 33:423–430, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903571358

DEVELOPMENT OF LLE AND SPE PROCEDURES AND ITS APPLICATIONS FOR DETERMINATION OF OLMESARTAN IN HUMAN PLASMA USING RP-HPLC AND HPTLC

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□ *Two novel simple and rapid liquid–liquid and solid phase extraction methods have been developed for determination of olmesartan in human plasma using zidovudine as an internal standard. Liquid–liquid extraction from spiked human plasma samples was done using Dichloromethane: acetic acid (5.5: 0.5, v/v) solvent, while solid phase extraction was carried out on a DSC MCAX cartridge. For HPLC, the mobile phase consisted of water acetic acid pH 4.5 and methanol (25:75) at a flow rate of 0.5 mL · min^{-1} in isocratic mode. The calibration curve was plotted with a concentration range 10 μgmL^{-1} to 60 μgmL^{-1}. Recovery studies were carried out by LLE and SPE procedures with average recoveries 69.27% and 72.87%, respectively. For HPTLC the developing phase consisting of ethyl acetate methanol and acetic acid (8.0:2.0:0.05 v/v/v) and detection was carried out on 269 nm. The calibration curve was plotted over the concentration range of 200 ng to 600 ng. The average recoveries were 90.12% and 79.64% by LLE and SPE, respectively. The proposed method was validated as per US-FDA guidance.*

Keywords HPTLC, human plasma, liquid–liquid extraction, Olmesartan Medoxomil, RP-HPLC, solid phase extraction

INTRODUCTION

Olmesartan Medoxomil, a prodrug of Olmesartan, is a selective AT1 subtype angiotensin II receptor antagonist. It blocks the vasoconstrictor effect of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in the vascular smooth muscle. Chemically it is 1-((2′-(1*H*-tetrazol-5-yl) biphenyl-4-yl) methyl)-2-butyl-4-(2-hydroxypropan-2-yl)-4, 5-dihydro-1*H*-imidazole-5-carboxylic acid.[1–4] A literature survey revealed that several analytical methods were reported for the determination

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of Olmesartan,[5–9] in biological fluids including liquid chromatography tandem mass spectrometry.[10–11] The objective of the present study was to develop extraction procedures for Olmesartan from human plasma and its estimation by using RP-HPLC and HPTLC, and validate the developed method as per USFDA guidance.

EXPERIMENTAL

Materials

Olmesartan Medoxomil was supplied by Ajanta Pharma Ltd. (Paithan, India). Zidovudine was received from Emcure Pharma Ltd. (Pune, India). The methanol, glacial acetic acid, ammonia (liquor), acetonitrile and dichloromethane were procured from Qualigens Fine Chemicals Ltd. Ethyl acetate was purchased from S d Fine Chem. Ltd. (Mumbai, India). Drug free human plasma was procured from Arpan Blood Bank (Nashik, India).

Instrumentation

The HPLC system was equipped with binary pumps Smartline-1000–1, 2, and Smartline-UV-2600 data were acquired and processed using Chromgate 3.1 software (all were from Knauer, Berlin, Germany). For HPTLC, the samples were applied using Linomat V sample applicator with a Camag 100 µL syringe. The densitometry scanning was performed by using a Camag TLC scanner III supported by win CATS software (Camag, Muttenz, Switzerland).

Chromatographic Conditions

HPLC measurements were carried out using a reverse phase Eurosphere-100 C18 (250 mm × 4.6 mm × 5µ) column operated at ambient temperature isocratically at 0.5 mLmin^{-1} with mobile phase water acetic acid (pH 4.5) and methanol (25:75, v/v). Detection was carried out at 254 nm injection volume 20 µL. The Chromatogram of Olmesartan and Zidovudine is shown in Figure 1.

Chromatographic separation with HPTLC was performed using a precoated silica gel plate 60 F_{254} (20 cm × 10 cm) with 250 µm thickness. Development was carried out in a 20 cm × 10 cm twin trough glass chamber with the mixture of ethyl acetate, methanol and acetic acid in the ratio of (8.0:2.0:0.05 v/v/v) as the developing solvent. Time for chamber saturation was optimized to 10 min. The length of chromatographic development was 70 mm. The densitometry scanning was performed at 269 nm. Figure 2 shows the HPTLC chromatogram of Olmesartan and Zidovudine.

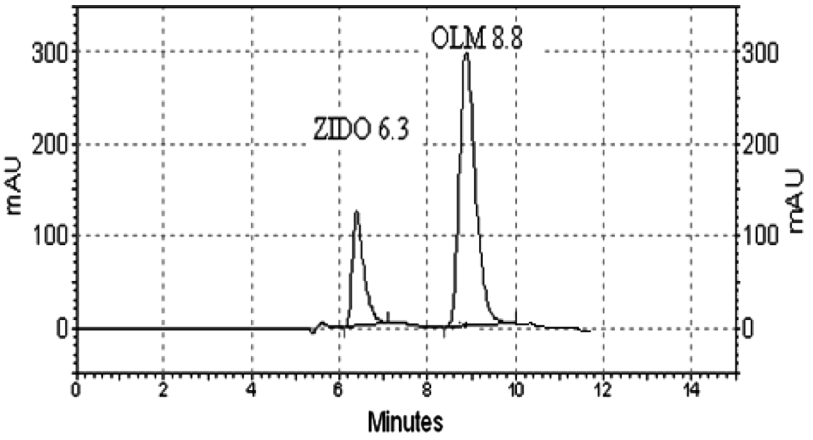

FIGURE 1 Chromatogram of pure Olmesartan (t_R6.8) and Zidovudine (t_R8.8).

Calibration Standard (CS) and Quality Control (QC) Samples in Human Plasma

For HPLC in each 15 mL centrifuge tube, the stock solution (1.0 mgmL^{-1}) was spiked in volume of 0, 10, 20, 30, 40, 50, 60 µL to drug free human plasma to provide calibration standards in the concentration range equivalent to 0 (no Olmesartan added, i.e., blank), 10, 20, 30, 40, 50, 60 µgmL^{-1} and the volume of internal standard (Zidovudine) was kept constant 30 µgmL^{-1}.

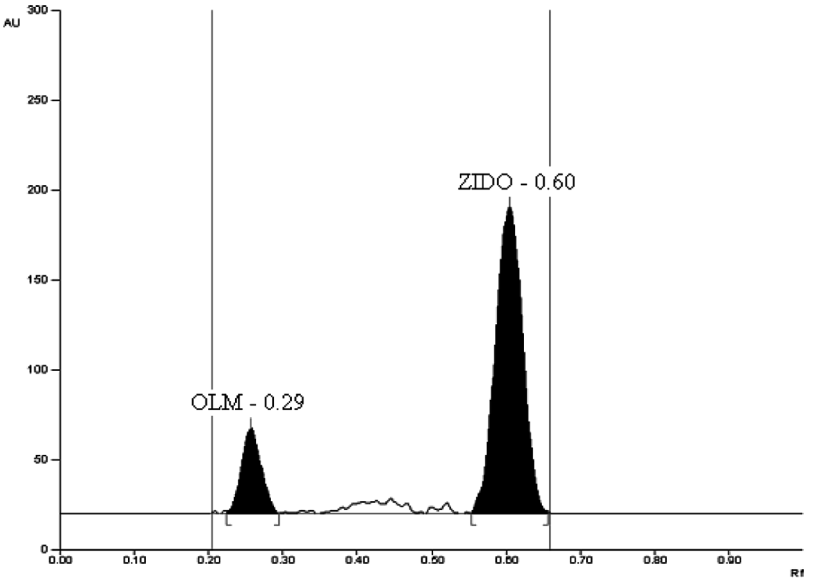

FIGURE 2 Chromatogram of pure Olmesartan (R_f 0.29) and Zidovudine (R_f 0.60).

The quality control samples were prepared in plasma in the concentration range of 30, 40, 50 μgmL^{-1} and kept for 30 min.

After 30 min, for LLE in CS and QC samples, protein precipitation was done with acetone and extracted using 5.5 mL dichloromethane and 0.5 mL acetic acid as extracting solvent. For the SPE technique, 0.5 mL acetic acid was added in each tube to avoid anion formation. For protein precipitation 2 mL acetonitrile was added in each tube.

Further, these samples were vortexed on a mixer for 1 min and then centrifuged at 10,000 rpm for 5 min.

In the LLE procedure, the organic phase was recovered and evaporated to dryness in a water bath at 50°C. The mass was reconstituted with 1 mL mobile phase. In SPE, the supernatant mixture after centrifugation was loaded to DSC MCAX SPE column (1 g), which was pretreated with 1 mL of methanol first, followed by 1 mL of acetic acid (pH 2.6). The column was vacuumed to dryness and the analytes were eluted with 1 mL of 10% ammonium hydroxide in methanol.

For HPTLC, in a 15 mL centrifuge tube the volume from the stock solution (1 $mgmL^{-1}$) containing 0, 20, 50, 75, 100, 125, and 150 µg of Olmesartan Medoxomil and 100 µg of Zidovudine as an internal standard were spiked to drug free human plasma. The quality control (QC) samples were prepared in plasma by spiking 50, 100, and 150 µg and kept for 30 min; LLE and SPE procedures after 30 min were carried out as explained for HPLC.

RESULT AND DISCUSSION

Method Validation

The proposed method was validated for selectivity, sensitivity, accuracy, precision, recovery, linearity, and stability according to the USFDA Guidance for the validation of bioanalytical methods.[12,13] The summary of all validation parameters is shown in Table 1.

Selectivity

Interfering peaks were not observed in the chromatogram of blank pooled human plasma. Typical chromatograms were obtained from drug free human plasma (blank sample) and plasma sample spiked at lower limit of quantitation (LLOQ) (80 ng) with internal standard.

Sensitivity

The accuracy and precision at the lower limit of quantitation (LLOQ) was analysed by using five replicates of the sample. Peak response was

TABLE 1 Summary of Validation Parameters

Parameters	HPLC		HPTLC	
	LLE	SPE	LLE	SPE
Linearity range	10–60 $\mu g mL^{-1}$		80–600 ng	
Correlation co-efficient	0.9690	0.9630	0.9900	0.9820
LLOQ	10 $\mu g mL^{-1}$		80 ng	
Extraction				
Efficiency (% Recovery)	69.27	72.87	90.12	79.64
Accuracy (% RE)				
i. Low	1.00	9.56	11.89	6.76
ii. Mid	12.60	3.65	2.53	3.83
iii. High	1.72	12.88	0.65	7.14
Precision (CV)				
1. Inter day				
i. Low	3.75	2.29	3.29	3.00
ii. Mid	2.01	1.56	1.02	1.51
iii. High	0.74	0.84	1.11	0.69
2. Intra day				
i. Low	5.02	2.94	2.59	2.86
ii. Mid	2.64	1.04	1.07	1.13
iii. High	0.89	0.78	1.04	0.72

considered for the calculations and it was determined as peak area ratio. The sensitivity is determined by % relative error and coefficient of variance at LLOQ (80 ng).

Accuracy and Precision

Accuracy and precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) for the set of quality control samples (low, mid, high) in replicate. The results revealed excellent intra and inter day accuracy and precision of the method, which is within the acceptable limit.

Extraction Efficiency

Absolute recovery was calculated by comparing peak areas obtained from freshly prepared samples extracted with unextracted standard solutions of the same concentration. Recovery data was determined in triplicates at three concentrations as recommended by the FDA guidelines.[12] Results showed the satisfactory extraction efficiency of Olmesartan by LLE and SPE from human plasma. The results also confirmed the reproducibility of the method.

TABLE 2 Linearity and Calibration Data of Olmesartan by HPLC Method ([c]n = 5)

Concentration (μgmL^{-1})	Mean Peak Response ± [a]S.D., ± [b]R.S.D. (%)	
	LLE	SPE
10	0.4602 ± 0.032425, ± 7.045	0.8849 ± 0.04778, ± 5.339
20	0.8801 ± 0.01679, ± 1.908	2.2418 ± 0.040277, ± 1.797
30	1.2509 ± 0.056721, ± 4.535	3.5590 ± 0.105109, ± 2.953
40	1.7821 ± 0.045834, ± 2.572	4.2236 ± 0.044721, ± 1.059
50	1.9966 ± 0.01837, ± 0.920	4.8083 ± 0.035493, ± 0.738
60	2.1373 ± 0.012019, ± 0.562	7.0880 ± 0.176063, ± 2.484

[a]S.D. = Standard deviation.
[b]R.S.D. = Relative standard deviation.
[c]n = Number of determination.

Linearity

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The linearity of Olmesartan was determined at five concentration levels ranging from 10 to 60 μgmL^{-1} and 80 to 600 ng for HPLC and HPTLC, respectively. The results are shown in Tables 2 and 3. The linear regression equations of the lines are:

$$\begin{aligned} \text{HPLC} \quad & y = 0.035x + 0.190, \quad (r^2 = 0.9690) \text{ by LLE} \\ & y = 0.112x - 0.144, \quad (r^2 = 0.9630) \text{ by SPE} \\ \text{HPTLC} \quad & y = 0.002x + 0.087, \quad (r^2 = 0.9900) \text{ by LLE} \\ & y = 0.003x + 0.262, \quad (r^2 = 0.9820) \text{ by SPE} \end{aligned}$$

TABLE 3 Linearity and Calibration Data of Olmesartan by HPTLC Method ([c]n = 3)

Concentration (ng)	Mean Peak Response ± [a]S.D., ± [b]R.S.D. (%)	
	LLE	SPE
80	0.2303 ± 0.00502, ± 2.180	0.4299 ± 0.001131, ± 0.263
200	0.5415 ± 0.005374, ± 0.992	0.9464 ± 0.00396, ± 0.417
300	0.7486 ± 0.013081, ± 1.748	1.2600 ± 0.002192, ± 0.174
400	1.0203 ± 0.001414, ± 0.139	1.5238 ± 0.031891, ± 2.062
500	1.2638 ± 0.007425, ± 0.588	1.9571 ± 0.017395, ± 0.894
600	1.5622 ± 0.010253, ± 0.656	2.4499 ± 0.084146, ± 3.353

[a]S.D. = Standard deviation.
[b]R.S.D. = Relative standard deviation.
[c]n = Number of determination.

TABLE 4 Stability of Olmesartan ([c]n = 3)

Stability (% Recovery Differences from Fresh Extract)	HPLC		HPTLC	
	LLE	SPE	LLE	SPE
Bench top				
i. Low QC	−2.10	−7.90	−9.61	4.49
ii. High QC	−5.79	−2.16	−2.31	0.10
Freeze thaw				
i. Low QC	−2.89	−8.10	−0.15	7.78
ii. High QC	−6.38	−1.02	−2.81	2.23
Post preparative				
i. Low QC	−3.63	1.14	−1.06	4.92
ii. High QC	−6.47	−2.09	−2.33	0.54

[c]n = Number of determination.

Stability

Stability of Olmesartan in plasma at various conditions was evaluated at low and high QC concentrations. Stability presented by calculating the difference of the percentage recoveries between freshly prepared samples and that of quality control samples are shown in Table 4.

CONCLUSION

The proposed HPLC and HPTLC methods were simple, rapid, accurate, and precise for estimation of Olmesartan from human plasma. Both extraction procedures give satisfactory and reproducible recovery of Olmesartan from human plasma. Among these two extraction procedures, LLE is more suitable as it gives high recovery as compared to SPE.

ACKNOWLEDGMENTS

We acknowledge Ajanta Pharma Ltd. (Paithan, India) and Emcure Pharma Ltd. (Pune, India) for providing gift samples of Olmesartan Medoxomil and Zidovudine, respectively. We would like to thank Arpan Blood Bank, (Nashik, India) for providing human plasma for the research work. Authors are thankful to Prof. V. M. Aurangabadkar, Principal, M. G. V.'s Pharmacy College, Nashik for providing the necessary facilities for the research work.

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