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Determination of plasma concentrations of losartan in patients by HPLC using solid phase extraction and UV detection☆

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Abstract

Purpose: To establish a HPLC assay for plasma losartan and its active metabolite EXP3174 to facilitate clinical pharmacokinetic studies. *Methods:* the HPLC system consisted of a 250×2 mm i.d. C_{18} reversed phase column preceded by a 4×4 mm guard column, a UV detector set at 254 nm, and an integrator. The mobile phase was a mixture of 0.01 M ammonium phosphate: acetonitrile: methanol (6:3:1) containing 0.02% sodium azide and 0.04% TEA, with pH adjusted to 3.2. The system was operated isocratically at ambient temperature at a flow rate of 0.3 ml/min. Losartan and its active metabolite EXP3174 were extracted from plasma using C_2 bonded silica gel standard solid phase extraction. *Results:* recoveries of losartan and EXP3174 from plasma were greater than 70%. Using 0.5 ml of plasma sample, standard curves were linear from 10 to 300 ng/ml ($r^2 = 0.996$ and 0.997 for losartan and EXP 3174, respectively). Sensitivity of the assay was < 10 ng/ml. Intra-and inter-assay variations were < 10 and 15%, respectively. The assay has been successfully applied to measuring plasma concentrations of losartan and EXP3174 in patients receiving a daily dose of losartan (50–100 mg). *Conclusion:* The HPLC assay has adequate sensitivity, reproducibility, and specificity for clinical pharmacokinetic studies.

Keywords: Losartan; EXP3174; HPLC; Plasma; Pharmacokinetics; Metabolism

1. Introduction

Losartan is an angiotensin II receptor antagonist for the treatment of hypertension and related disorders Medical Letter (1998). It is metabolized in the body to a pharmacologically active carboxlyic acid metabolite EXP 3174 (Lo et al., 1995),

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which is about five time more potent and has longer elimination half-life ($t_{1/2}$) than losartan (Munafo et al., 1992; Lankford et al., 1997). Many potential drug interactions have been reported for losartan which is metabolized mainly by the CYP2C9 and CYP3A4 isozymes (Kazierad et al., 1997; Kaukonen et al., 1998; Williamson et al., 1998). In order to understand the significance of these drug interactions, plasma concentrations of losartan and its active metabolites EXP 3174 should be measured under the clinical situation in questions. This has been most commonly attained by HPLC (Farthing et al., 1997; Soldner et al., 1998). The current paper describes the development and validation of a HPLC assay using solid phase extraction and UV detector for measurement of losartan and EXP3174 in plasma obtained from patients treated chronically with losartan.

2. Materials and methods

2.1. Chemicals

Losartan, its metabolite EXP3174, and the internal standard L-158,809 were kindly received as gifts from Merck & Co. Canada, Inc. (Point Claire, QC, Canada). Solvents were HPLC grade (BDH Chem., Halifax, NS, Canada), and all the other chemicals were reagent grade (Fisher Scientific, Ont., Canada).

2.2. HPLC system and operating conditions

The HPLC system consisted of a solvent delivery module (Beckman 114 M, Berkeley, CA, USA), a Rheodyne syringe loading injector (model 9725) with a 100 ul PEEK injection loop (Scientific Products & Equipment, Concord, Ont., Canada), a 5 μm 300 Å 250×2.0 mm i.d. C_{18} reversed phase analytical column (Jupiter®, Phenomenex, Torrance, CA 90501, USA) with a 5 μm 4.0×4.0 mm i.d. C_{18} reversed phase cartridge quard column (Licrocart, E.M. Merck, Germany), a Shimadzu Model SPD-6AV variable wavelength UV–VIS spectrophotometric detector with wavelength set at 254 nm (Man-Tech Assoc. Inc., Guelph, Ont., Canada), and a Beckman Model 427 Computing Integrator (Berkeley, CA, USA). The mobile phase consisted of 60% 0.01 M $NH_4H_2PO_4$ containing 0.02% sodium azide, 30% acetonitrile, 10% methanol, 0.04% triethylamine (TEA) with pH adjusted to 3.2. It was operated at room temperature under isocratic condition with a flow rate of 0.3 ml/min at a pressure between 3.3–3.8 kpsi. A system check was performed before and at the end of each run using a standard mixture of losartan, EXP 3174 and the internal standard L-158,809 (5 ug/ml each).

2.3. Solid phase extraction

Plasma samples or standards (0.5 ml) were applied to pre-conditioned 500 mg C_2 SPE columns (Chromosep® columns, Chromatographic Specialties Inc., Ont., Canada). Each column was then washed twice with water (1.0 ml each), and then with methanol (2×0.5 ml) to recover the analytes. The methanol extract was dried under a stream of N_2 at 55°C. The samples were stored at -20°C until analysis.

2.4. Clinical samples

Blood samples were collected from hypertensive patients treated in the Department of Medicine, QEII Health Sciences Centre, who received 50–100 mg of losartan (Cozaar®, Merck and Co. Canada, Point Claire, QC) daily for more than 1 week. Some of them also received other medications such as verapamil, furosemide, warfarin, alprazolam and thyroxin. Each blood sample was taken approximately 3 h after the last dose of losartan. Each collected sample was immediately centrifuged (3000 rpm, 4°C, 10 min) to separate the plasma, which was stored at -70°C until analysis.

2.5. Data analysis

Peak height ratios were recorded from the peak height of losartan or EXP 3174 to that of the internal standard L-158,809. Standard curves were constructed using simple linear regression. Each standard concentration was assayed in quadruplicate.

3. Results

Losartan, EXP 3174 and L-158-809 (Fig. 1) were well separated by the HPLC under the described condition (Fig. 2). Retention times ranged from 8 min for the internal standard L-158,809 to 20 min for EXP3174. A list of drugs which may be prescribed with losartan did not interfere with the assay (Table 1). Based on the results obtained from on-column injection and a signal to noise ratio of three, the limit of detection under the described condition was about 1 ng for both the analytes. The limit of quantitation was 10 ng/ml at which the coefficient of variation (CV) was 8% for losartan and 7% for EXP 3174, respectively. Using plasma concentrations of 10 and 100 ng/ml, recoveries of the analytes were about 97% for losartan and 76% for EXP3174. Standard curves were linear from 10–300 ng/ml for both losartan and EXP3174 ($r^2 > 0.99$) (Table 2). Sensitivity of the assay was < 10 ng/ml using 0.5 ml of plasma sample. The slopes and intercepts of each standard curve obtained from five independent HPLC assay run over a 2 month period is presented in Table 3, and their differences were less than 20%. Intra-assay and inter-assay variations were $< 15\%$ as determined over a 2-month period using quality control samples made up at 10, 50, and 100 ng/ml (Table 4).

Plasma concentrations of losartan and EXP3174 were measurable in all the samples collected from nine patients receiving 50–100 mg losartan daily. Mean plasma concentrations of losartan and EXP 3174 at 3 h after the last dose were 29 ± 20 and 65 ± 46 ng/ml, respectively ($n = 9$).

4. Discussion

During the method development, it was found that separation of losartan, EXP3174 and the internal standard L-158,809 was highly pH dependent. The retention time of EXP3174 was sensitive to the changes in pH of the mobile phase particularly when it was between 3 and 5. On the other hand, losartan and the internal standard were less affected at this pH range. It was determined that maximum separation occurred at pH about 3.2. For this reason, it was used as the operating pH of the mobile phase. In addition to the effect of pH, the composition of organic modifier also affected the separation. Increasing the relative content of methanol or decreasing that of acetonitrile in the mobile phase can drastically increase the retention time of the analytes and internal standard. The current mobile phase composition was selected because it allowed separation of the analytes from the endogenous plasma substances, and that each single run can be completed in 20 min. The current method is different from the previously reported HPLC assays (Furtek and Lo, 1992; Williams et al., 1996; Soldner et al., 1998). Both Furtek, Soldner and their co-workers used cyano-bonded HPLC columns (3 or 5 μm, 250 × 4.6 mm i.d. UltremexTM from Phenomenex, Tor-

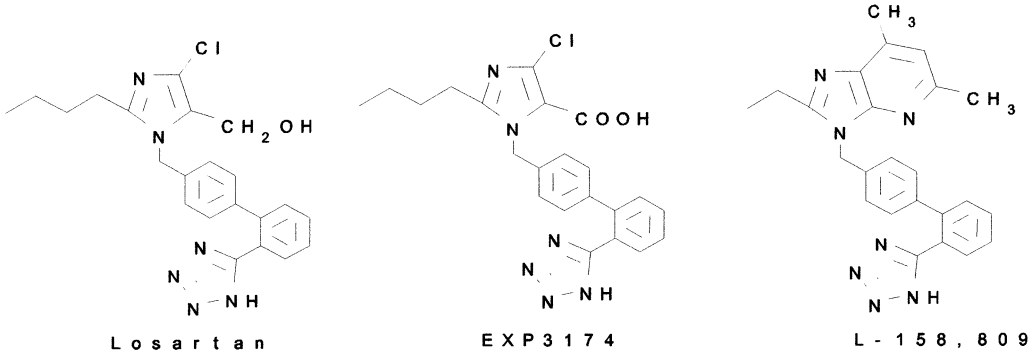

Fig. 1. Chemical structures of losartan, EXP 3174 and the internal standard L-158,809.

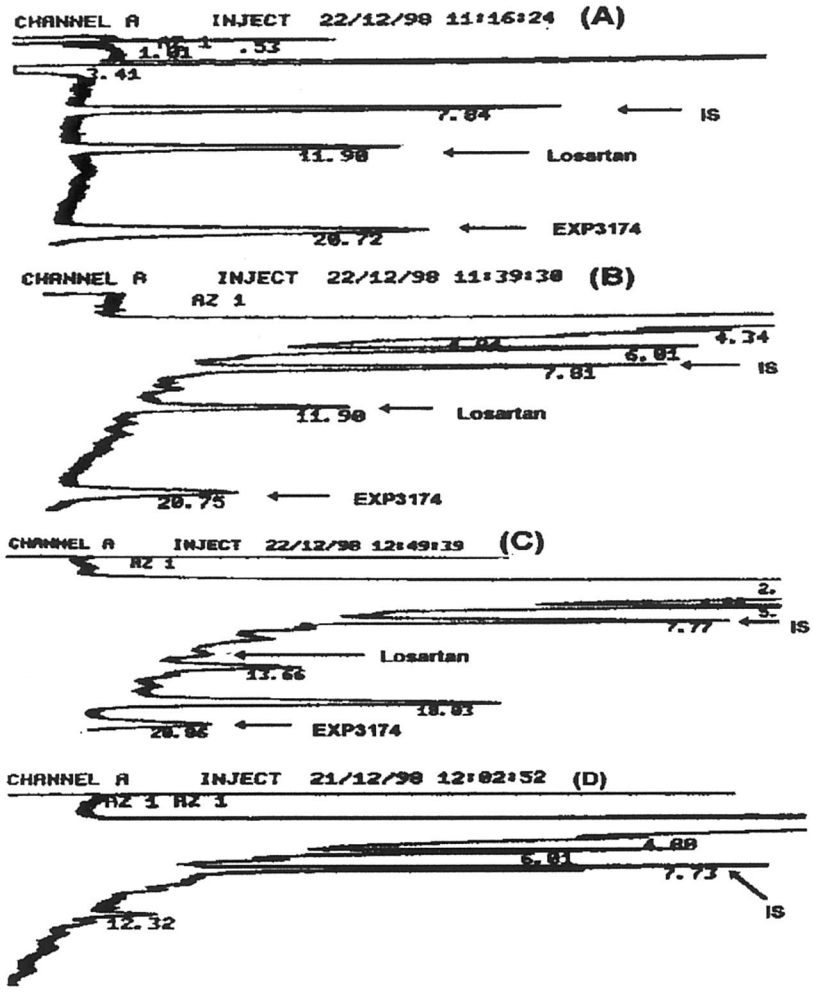

Fig. 2. HPLC-UV chromatograms of losartan, EXP3174, and the internal standard L-158,809. (A) Standard stock solution (5 ng each); (B) 100 ng/ml plasma standard; (C) plasma sample taken from a patient who received 50 mg losartan daily; and (D) plasma blank.

Table 1
Retention times of losartan, EXP 3174, L-158,809 and drugs tested for interference

Chemical agent tested	Retention time (min)	Chemical agent tested	Retention time (min)
Losartan	12	Nifedipine	17
EXP3174	20	Naproxen	18
L-158,809 (IS)	8.4	Verapamil	22
Dipyridamole	5.6	Chlorimpramine	22
Propranolol	5.7	Indomethacine	>40
Nortrityline	12	Metronidazole	>40
Ketoprofen	16	Ampicillin	>40

Table 2
Typical standard curves of losartan and EXP 3174[a]

Losartan concentration (ng/ml)	Peak height ratio	EXP 3174 Concentration (ng/ml)	Peak height ratio
300	4.0 ± 0.27	300	3.9 ± 0.14
100	1.2 ± 0.04	100	1.3 ± 0.09
50	0.81 ± 0.06	50	0.88 ± 0.11
10	0.26 ± 0.02	10	0.27 ± 0.02
Blank	0.0	Blank	0
Slope	0.013	Slope	0.013
Intercept	0.11	Intercept	0.068
r^2	0.996	r^2	0.997

[a] Each value represents mean $\pm$ S.D. of four independent determinations.

rance, CA, USA), and reported a separation of losartan, EXP3174, and their internal standard L-158,854 in plasma samples in less than 20 min (Furtek and Lo, 1992; Soldner et al., 1998). On the other hand, Williams and his co-workers (Williams et al., 1996) have used 4.6×250 mm reversed phase C8 column (Lichrosorb 10 from Phenomenex, Torrance, CA, USA) to quantitate losartan (retention time 12 min) in tablets (Cozaar®, Merck & Co. Inc.). The current HPLC assay uses a 2×250 mm C_{18} reversed phase minibore column which consumes considerably less mobile phase (0.3 vs. 1 ml/min), increases resolution between losartan and EXP3174 (resolution factor 2.7 vs. 0.67), without significantly increasing the time needed for each HPLC run (about 20 min).

Previous workers have reported good recoveries (70–90%) of losartan and its metabolites EXP3174 from plasma using liquid liquid extraction with methyl *tert*-butyl ether (MTBE) and cyano bonded solid phase extracton columns (Furtek and Lo, 1992; Soldner et al., 1998). The current method uses 500 mg C_2 solid phase extraction columns which is a less labour intensive procedure than the liquid liquid extraction, but also able to effectively extract these compounds from plasma with $>70\%$ recovery. It was founded that the use of 100 mg C_2 or C_{18} solid phase extraction columns resulted in considerably lower and variable recoveries. Sensitivity of the current method is <10 ng/ml which is similar to the previously described procedures (Furtek and Lo, 1992; Williams et al., 1996; Soldner et al., 1998). However, it should be noted that the standard curve data were curvilinear between 10 and 50 ng/ml, which could compromise the results at the lower conentrations. Despite these potential drawbacks, the accuracies were within 50 and 25% at 10 ng/ml, and less than 10% at 100 ng/ml for losartan and EXP 3174, respectively. The r^2 of the standard curves were >0.99 for both the analytes (Tables 2 and 5).

In summary, the current method is a simple HPLC procedure which has been validated for linearity, sensitivity, precision and interference caused by endogenous plasma materials or other medications. The method has been applied successfully to measure plasma losartan and EXP3174 concentrations in patients chronically receiving losartan.

Table 3
Standard curve statistics over a period of 2 months

HPLC run	Slope		Intercept	
	Losartan	EXP 3174	Losartan	EXP 3174
1	0.013	0.013	0.068	0.11
2	0.010	0.011	0.053	0.068
3	0.011	0.010	0.063	0.087
4	0.0093	0.010	0.056	0.076
5	0.012	0.012	0.045	0.069
Mean	0.011	0.011	0.057	0.081
S.D.	0.0014	0.0011	0.0086	0.016
%CV	12.6	9.7	15.2	19.4

Table 4
Intra- and inter-assay variations of the HPLC method[a]

Analytes	Concentrations (ng/ml)	Mean intra-assay variations (%CV)	Mean inter-assay variations (%CV)
Losartan	10	4.1	12
	100	7.9	9.0
EXP 3174	10	9.8	13
	100	4.2	4.1

[a] Values were determined over a 2-month period including the analysis of four sample batches.

Table 5
Steady-state plasma concentrations of losartan and EXP 3174 in patients receiving 50–100 mg OD of losartan (Cozaar)

Patient no.	Losartan (ng/ml)	EXP 3174 (ng/ml)
1	18	7.9
2	12	89
3	20	77
4	4.7	20
5	16	163
6	43	55
7	63	84
8	48	55
9	40	33
Mean ± S.D.	29 ± 20	65 ± 46

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References

Farthing, D., Sica, D., Fakhry, I., Pedro, A., Gehr, T.W., 1997. Simple high-performance liquid chromatographic method for determination of losartan and E-3174 metabolite in human plasma, urine and dialysate. J. Chromatogr. B Biomed. Sci. Appl. 704, 374–378.

Furtek, C.I., Lo, M.W., 1992. 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. (Biomed. Appl.) 573, 295–301.

Kaukonen, K.M., Olkkola, K.T., Neuvonen, P.J., 1998. Fluconazole but not itraconazole decreases the metabolism of losartan to E-3174. Eur. J. Clin. Pharmacol. 53, 445–449.

Kazierad, D.J., Martin, D.E., Blum, R.A., Tenero, D.M., Ilson, B., Boike, S.C., Etheredge, R., Jorkasky, D.K., 1997. Effect of fluconazole on the pharmacokinetics of eprosartan and losartan in healthy male volunteers. Clin. Pharmacol. Ther. 62, 417–425.

Lankford, S.M., Plummer, D., Hellyer, P., Christ, D.D., Bai, S.A., 1997. Pharmacokinetic-pharmacodynamic relations of losartan and EXP3174 in a porcine animal model. J. Cardiovasc. Pharmacol. 30, 583–590.

Lo, M.W., Goldberg, M.R., McCrea, J.B., Lu, H., Furtek, C.I., Bjornsson, T.D., 1995. Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP3174 in humans. Clin. Pharmacol. Ther. 58, 641–649.

Medical Letter, 1998. Candesartan for hypertension. The Med. Lett. 40, 109–110.

Munafo, A., Christen, Y., Nussberger, J., Shum, L.Y., Borland, R.M., Lee, R.J., Waeber, B., Biollaz, J., Brunner, H.R., 1992. Drug concentration response relationships in normal volunteers after oral administration of losartan, an angiotensin II receptor antagonist. Clin. Pharmacol. Ther. 51, 513–521.

Soldner, A., Spahn Langguth, H., Mutschler, E., 1998. 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, 863–873.

Williams, R.C., Alasandro, M.S., Fasone, V.L., Boucher, R.J., Edwards, J.F., 1996. Comparison of liquid chromatography, capillary electrophoresis and super-critical fluid chromatography in the determination of losartan postassium drug substance in Cozaar tablets. J. Pharm. Biomed. Anal. 14, 1539–1546.

Williamson, K.M., Patterson, J.H., McQueen, R.H., Adams, K.F., Pieper, J.A., 1998. Effects of erythromycin or rifampin on losartan pharmacokinetics in healthy volunteers. Clin. Pharmacol. Ther. 63, 316–323.