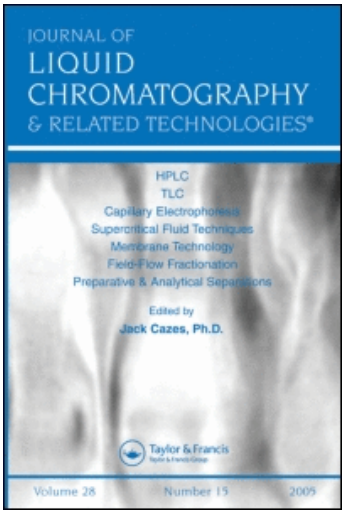

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HPLC DETERMINATION OF UTIBAPRIL AND ITS DIACID FPL 63674XX IN RODENT LABORATORY DIET USING SELECTIVE EXTRACTION AND GRADIENT ELUTION CHROMATOGRAPHY

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ABSTRACT

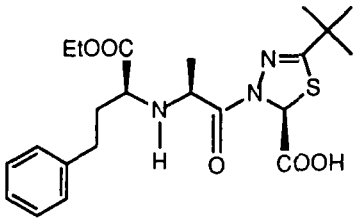
Utibapril is a novel thiadiazoline that is currently under investigation as an antihypertensive agent. The major degradation product, a diacid FPL 63674XX, is biologically active. An HPLC method has been developed to simultaneously determine both utibapril and its diacid in rodent laboratory diet used to dose animals in long term toxicology studies. The method is based on liquid-solid extraction of the compounds followed by direct injection of the extract. Gradient elution chromatography is utilized to better separate the diacid from interferences. Recovery of utibapril and the diacid from the lab diet was found to be 97.6 $\pm$ 0.44% and 99.7 $\pm$ 2.8%, respectively (n = 3) at 0.43% w/w levels. The separation is achieved on a octadecylsilane column using a flow rate of 0.75 ml/min and a column temperature of 70°C. The UV detector was set at 260 nm. A linear and step gradient program was established using mobile phases consisting of 0.05 M

phosphate buffer, pH 2.5 and 40:60 0.05 M phosphate buffer, pH 2.5 - acetonitrile. An injection volume of 20μl was utilized and the chromatographic run time was set at 38 min. The assay method shows linearity for utibapril over a 0.25 - 2% w/w range ($r = 0.9998$, $n = 5$) and for the diacid over a 0.0043 - 0.43% w/w range ($r = 0.9960$, $n = 5$). Accuracy and precision for both compounds were in the 1-5% range over the linear ranges specified above. The method was applied to an actual rodent diet sample formulated to contain 0.43% w/w utibapril.

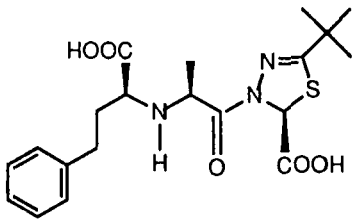
INTRODUCTION

Utibapril, a novel thiadiazoline, (2,3-Dihydro-3-[N-(1S)-(1-ethoxycarbonyl-3-phenylpropyl)-L-alanyl]-5-(1,1-dimethylethyl)-1,3,4-thiadiazole-2-(S)-carboxylic Acid) is a new angiotensin converting enzyme inhibitor currently under investigation as an antihypertensive agent. Utibapril is the ester prodrug of the biologically active diacid FPL 63674XX, which is also the major degradation product [1-3]. Both structures are shown in Figure 1.

Admixtures of utibapril and animal feeds provide the simplest and most economical route for dosing animals in long-term toxicology studies. To validate such studies, confirmation of drug levels in the diet is necessary. In addition, homogeneity of mixing and stability of the drug in the animal feed are required to satisfy good laboratory practices guidelines. High Performance Liquid Chromatography (HPLC) is well adapted to the assay of drugs in diet admixtures; chromatographic systems have been shown to provide good separations of analytes from feed extractibles. Three methods have been used in the development of



UTIBAPRIL



FPL 63674XX

Figure 1 - Structures of utibapril and its major degradation product, the diacid FPL 63674XX.

HPLC assays for drugs in medicated feeds. They are direct injection of organic extracts, minimum cleanup using alumina columns, and extensive cleanup using multi-step extractions. A method is usually chosen based on levels of the drug in the sample. Lower concentrations of drug in the feed will require a more extensive cleanup procedure to isolate drug from the complex feed matrix. Direct injection of diet extracts is the usual assay method found in the literature. Diacetolol hydrochloride [4], methapyrilene hydrochloride [5], labetalol [6] and sulfamethazine [7]

have been analysed using this approach. Methods involving minimum cleanup of the feed using alumina columns have included furazolidone [8], nitrofurazone and ethopabate [9] and 1-(2-aminoethyl)-3-(2,6-dichlorophenyl)thiourea [10]. Extensive cleanup has been used in the HPLC procedures for arprinocid reported by Fink [11], diethylstilbestrol reported by King et al [12], and an assay for vitamin E by Cohen and Lapointe [13] which included saponification and filtration, followed by evaporation to dryness before dissolution and chromatography. Other methods using extensive sample cleanup include a reverse phase HPLC determination of melengestrol acetate in feed supplements [14] and post column derivatization of clarified extracts of sulfamethazine and sulfathiazole for assay of the drugs in feeds and feed premixes [15].

In this paper, an HPLC method to determine utibapril and the diacid in rodent laboratory diet based on direct injection of feed extracts is presented. The analytes are extracted from the diet with methanol-water, and are quantitated using gradient elution reversed phase chromatography.

MATERIALS AND METHODS

Reagents

Potassium dihydrogen phosphate was purchased from J.T. Baker (Phillipsburg, NJ, USA) and Phosphoric acid was purchased from EM

Science (Cherry Hill, NJ, USA). All other solvents were HPLC grade and were used without further purification.

Apparatus

The chromatographic system consisted of a Model 600E system controller, a WISP Model 710B autosampler, a Model 991 Photodiode array detector, a steel column heater module, a Powermate SX Plus computer equipped with a Model 5200 printer plotter; all from Waters Associates (Milford, MA,USA). A Waters Novapak octadecylsilane column (4μm, 300 X 3.9mm) was used.

The preparation of spiked samples using stock solutions was aided by the use of an electronic motorized pipette (EDP-Plus, Ranin Instrument Co., Woburn, MA USA).

Samples were filtered prior to injection using a Acrodisc CR PTFE 0.45 μm filter (Gelman Sciences, Ann Arbor, MI, USA).

A laboratory centrifuge (International Equipment Company, Boston, MA, USA) was used. Disposable centrifuge tubes (50mL) were obtained from Evergreen Scientific International, Inc. (Los Angeles, CA, USA).

Chromatographic conditions

Mobile Phase A consisted of 0.05 M Phosphate buffer, pH 2.5 and Mobile Phase B consisted of 40:60 0.05 M Phosphate buffer pH 2.5/acetonitrile. The analytes were separated on the octadecylsilane column using a flow rate of 0.75 ml/min and a column temperature of

70°C. The UV detector was set at 260nm. An injection volume of 20μl was utilized and the chromatographic run time was set at 38 min. The following gradient program was used:

Time, Min.	% A	% B	
0	80	20	
20	20	80	(Linear)
25	0	100	(Linear)
30	80	20	(Step)
35	80	20	

Preparation of Stock Solutions

A 1mg/ml stock solution of utibapril was prepared by dissolving 50 mg of the drug substance (Lot No PR 1047-661 FB, Fisons Pharmaceuticals) in 20:80 water/absolute methanol in a 50-ml volumetric flask. A 0.1 mg/ml stock solution of the diacid FPL 63674XX (Lot No PR 1047-668 FB, Fisons Pharmaceuticals) was also prepared in a similar fashion.

Preparation of spiked Utibapril/Diacid samples

Twenty grams of drug-free rodent laboratory diet pellets (Purina No. 5002 Rodent Chow) were blended to a homogeneous mixture with the aid of a Waring blender. Individually weighed one gram quantities of the blended pellets were transferred to 50-ml disposable centrifuge tubes into which were added 4.3, 8.5, and 17.0 ml of utibapril stock solution to give 0.43%, 0.85%, 1.70% w/w mixtures, respectively, and 43, 85, and 170 μl of the diacid FPL 63674XX stock solution to give 0.0043%,

0.0085%, 0.017% w/w mixtures, respectively. Each sample was then mixed for 15 sec on a Vortex Genie prior to assay.

Preparation of Assay Samples From Rodent diet pellets containing Utibapril

Rodent laboratory diet pellets formulated by Purina to contain 0.43%w/w utibapril were blended to a homogeneous mixture with the aid of a blender. An accurately weighed quantity (one gm) of the blend was then transferred to a 50-ml disposable centrifuge tube for assay.

Assay Method

Twenty ml of extraction solvent (20:80 distilled water/ methanol) was added to each centrifuge tube containing either spiked utibapril/ diacid or assay samples. Each mixture was vortexed for 5 sec, and placed on a wrist shaker for 12 min at 120 oscillations/min. After centrifugation for 6 min at 2500 R.P.M., the extract was decanted into a 100-ml volumetric flask. The extraction was repeated three times using fresh 20 ml portions of the extraction solvent. The extracts were collected in the volumetric flask and extraction solvent was added to volume. A 1 ml sample was filtered through a 0.45μm filter and 20 μl injected into the liquid chromatograph.

RESULTS AND DISCUSSION

The extraction of utibapril was investigated at the lowest expected level (0.43%w/w) of drug in the rodent lab diet. The recovery data is

TABLE 1

RECOVERY OF 0.43% w/w UTIBAPRIL FROM RODENT LABORATORY DIET USING DIFFERENT ORGANIC SOLVENTS.

Pretreatment Step	Extraction Solvent	% Recovery
Hexane[a]	Absolute Methanol	74.33 ± 3.29[b]
Hexane[a]	Acetonitrile	48.94 ± 0.13
--[c]	Absolute Methanol	80.5 ± 4.69
--[c]	Acetonitrile	81.08 ± 3.71
--[c]	Tetrahydrofuran	--[d]
--[c]	20:80 Distilled Water-Absolute Methanol	97.60 ± 0.44

[a] The feed was extracted with 3-20 ml hexane portions prior to the actual extraction step.
[b] Mean ± Standard deviation (n = 3).
[c] No pre-treatment step was performed.
[d] No measurable recovery of drug was obtained.

shown in Table 1. The highest extraction efficiency for the drug from the feed was obtained using a 20:80 mixture of water/methanol. Recovery was calculated to be 97.6 ± 0.44% (n=3). An identical extraction of the diacid FPL 63674XX from the feed matrix was found to give 99.7 ± 2.8% recovery (n=3).

Initially, the separation of utibapril and its diacid was attempted on phenyl (spherisorb phenyl, 5 um, 150 x 4.6 mm i.d.) and octadecylsilane (Novapak, 4 um, 150 x 3.9 i.d. and 300 x 3.9 mm i.d.) columns using a mobile phase consisting of 55:18:27 distilled water-absolute methanol-acetonitrile containing 1% perchloric acid. Retention times for utibapril and the diacid were 5 and 3 min, respectively. Upon

assay of the rodent diet extract, the diacid was found to co-elute with an unknown peak. The methanol and acetonitrile concentrations in the ternary mixture were then varied between ± 5% of the original mobile phase composition in efforts to effect separation of the diacid and interfering peak. None of these attempts allowed the resolution of the two peaks on either the phenyl or octadecylsilane columns. Binary mobile phases consisting of water/methanol and water/acetonitrile were also investigated in efforts to solve co-elution problem. In each case, the organic modifier concentration ranged from 17 to 50% v/v. These attempts were also unsuccessful. It was decided to investigate the use of gradient elution coupled with column heating to separate the two interfering peaks. After employing various solvent gradients and column temperatures(50-80°C), the gradient elution system described in the Materials and Methods section, coupled with a 70°C column temperature, was selected for use. Baseline resolution of the two previously interfering peaks was now obtained (Rs > 1.2). Retention times for utibapril and its diacid were 21.5 and 12.3 min, respectively. Typical chromatograms of a blank and spiked rodent diet sample are shown in Figure 2.

The assay method shows linearity for utibapril over the range 0.25%-2.0% w/w with a correlation coefficient of 0.9998 (n = 5). The diacid was linear over the range 0.0043-0.43% w/w with a correlation coefficient of 0.9960 (n = 5).

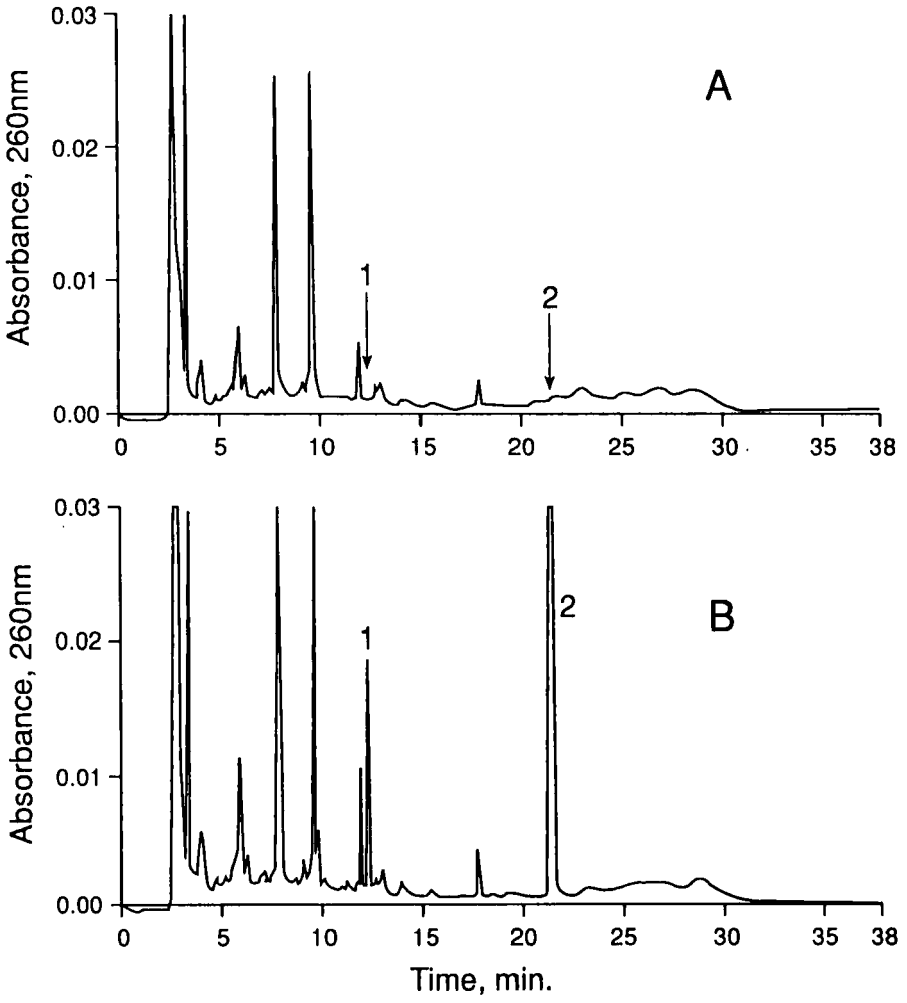


Figure 2 - Typical chromatograms of (A) blank and (B) rodent diet sample spiked with utibapril and its diacid. The separation was achieved on an octadecylsilane column at 0.75 ml/min flow rate and 70°C column temperature. The UV detector was set at 260nm. Gradient elution conditions are listed in Materials and Methods section of this paper. Key: 1, The diacid; 2, utibapril

TABLE 2
Accuracy and Precision of Spiked Samples of Utibapril and its Diacid in Rodent Laboratory Diet

Compound	% w/w Added	% Recovery	RSD (%)
Utibapril	0.43	95.44 ± 0.75[a]	0.79
	0.85	97.20 ± 0.79	0.81
	1.70	97.13 ± 1.59	1.63
Diacid	0.0043	96.72 ± 2.60	2.69
	0.0430	96.71 ± 1.27	1.31
	0.4300	97.15 ± 1.90	1.95

[a] Mean ± standard deviation based on n = 7

Accuracy and precision of the method were evaluated using rodent diet samples spiked with 0.43, 0.85 and 1.7% w/w of utibapril and 0.0043, 0.0430, and 0.430% w/w of the diacid. The data shown in Table 2 indicates that the method provides satisfactory accuracy (% recovery) and precision(%RSD) at the required concentration ranges.

The method was applied to an actual rodent diet sample commercially formulated to contain 0.43% w/w of utibapril. The sample was assayed by the method described herein and found to contain 0.41% w/w drug. The precision of the measurements was 1.60% (n=8). No detectable quantities of the diacid were found in this commercial sample.

In summary, a gradient elution reverse phase HPLC method has been developed for the assay of utibapril and its diacid in rodent

laboratory diet commercially formulated to contain utibapril. The use of gradient elution chromatography significantly reduces the sample preparation steps prior to analysis. The method was used for the monitoring of utibapril and its diacid levels in rodent laboratory diet samples used in long-term toxicology studies.

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REFERENCES

[1] O'Connor, S.E., ACE inhibitors-new developments., Drug News - Perspectives, 3, 33, 1990.

[2] Carr, R.D., Cooper, A.E., Hutchinson, R., Mann, J., O'Connor, S.E., Robinson, D.H. and Wells, E., Preferential biliary elimination of FPL 63547, a novel inhibitor of angiotensin-converting enzyme, in the rat., Brit. J. Pharmacol. 100, 90, 1990.

[3] Carr, R.E., Higgs, L., Killingback, P.G., Nicol, A.K., O'Connor, S.E., Robson, A., Wells, E. and Simpson, W.T., Pharmacological properties of FPL 63547, a novel inhibitor of angiotensin-converting enzyme., Brit. J. Pharmacol. 100, 83, 1990.

[4] Bowker, M.J., Gladwiz, R.P., Mills, D.J. and Stubbles, J.A., Semi-automated high-performance liquid chromatographic method for the dtermination of diacetolol hydrochloride in medicated animal diets., J. Chromatogr. 200, 261, 1980.

[5] Shaikh, B. and Hallmark, M.P., High performance liquid chromatographic determination of methapyrilene hydrochloride in feed and sleep aid tablets., J. Assoc. Off. Anal. Chem., 64, 889, 1981.

[6] Townley, E.R. and Ross, B., Paired-ion chromatography and high performance liquid chromatography of labetalol in feeds., J. Assoc. Off. Anal. Chem. 63, 1262, 1980.

[7] Cieri, W.R., High pressure liquid chromatographic dtermination of sulfmethazine or sulfathiazole and colorimetric dtermination of sulfathiazole in feeds:collaborative studies., J. Assoc. Off. Anal. Chem. 63, 727, 1980.

[8] Smallidge, R.L., Rowe, N.W., Wadgaonkae, N.D. and Stringham, R.W., High performance liquid chromatographic determination of Furazolidone in feed and feed premixes., J. Assoc. Off. Anal.Chem, 64, 1100, 1981.

[9] Thorpe, V.A., Sample preparation of carbadox, furazolidone, nitrofurazone, and ethopabate in medicated feeds for high pressure liquid chromatography., J. Assoc. Off. Anal. Chem, 63, 981, 1980.

[10] Quadrel, R.R. and Bogdansky, F.M., A. rapid chromatographic procedure for the isolation and quantitation of 1-(2-Aminoethyl)-3-(2,6-dichlorophenyl)thiourea from dietary admix followed by HPLC., J. Chromatogr. Sci, 18, 622, 1980.

[11] Fink, D.W., Liquid chromatographic method for determination of aprinocid in feed:collaborative study., J. Assoc. Off. Anal. Chem., 64, 973, 1981.

[12] King, J.R., Nony, C.R. and Bowman, M.C., Trace analysis of diethylstilbestrol [DES] in animal chow by parallel high-speed liquid chromatography, electron-capture gas chromatography, and radioassays., J. Chromatogr. Sci., 15, 14, 1977.

[13] Cohen, H. and Lapointe, M.R., Determination of vitamin E in animal feeds by normal phase high pressure liquid chromatography., J. Assoc. Off. Anal. Chem., 63, 1254, 1980.

[14] Roybal, J.E., High pressure liquid chromatographic dtermination of melengestrol acetate in dry feed supplements., J. Assoc. Off. Anal. Chem., 64, 661, 1981.

[15] Stringham, R.W., Mundell, E.C. and Smallidge, R.L., Use of post-column derivatization in liquid chromatographic determination of sulfamethazine and sulfathiazole in feeds and feed premixes., J. Assoc. Off. Anal. Chem. 65, 823, 1982.