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Note

High-performance liquid chromatography–ultraviolet method for the simultaneous determination of potential synthetic and hydrolytic impurities in urapidil fumarate

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Urapidil fumarate is an effective oral antihypertensive agent[1,2]. There have been several publications concerning the use of high-performance liquid chromatography (HPLC) to determine the metabolites and pharmacokinetics of urapidil[3–6]. The HPLC–UV procedure presented here evaluates urapidil fumarate for four synthetic precursors[7], two equimolar aqueous hydrolysis products, and three additional isolated and characterized components.

The method was designed to meet sensitivity requirements suggested by Wolters[8]. The limit of detection (LOD) and the limit of quantitation (LOQ) for each analyte were determined with 95% confidence by the method of Hubaux and Voss[9]. Interfaced computer programs were utilized to reduce the core data by the method of relative weight response (RWR)[10] and to determine the LODs and LOQs.

EXPERIMENTAL

Standard materials

Standard Analytical Reference Materials (SARMs) of the analytes, internal standard (IS), and urapidil fumarate were of purity $\geqslant$ 99%. See Fig. 1 for molecular structures.

Materials 1 and 4 were obtained by exhaustive hydrolysis of urapidil free base in 70°C aqueous 0.1 *N* hydrochloric acid with isolation as separate products using methylene chloride extraction before and after pH adjustment to 11 with sodium hydroxide. Compounds 2 and 5 are commercially available (Aldrich). The remaining SARMs were synthesized. Compounds 4 and 5 were prepared as the tri- and dihydrochloride salts, respectively, to prevent partial reaction with carbon dioxide during handling.

The IS was synthesized in particular for its structural similarity to the analytes and its resulting reproducible relative elution volume.

Standard solutions

A stock SARM mixture of the analytes was prepared in HPLC grade acetonitrile–water (9:1). The water was necessary to solubilize the hydrochloride salts but made at low content to impede hydrolysis of SARMs 3, 6, 7, and 8. This solution

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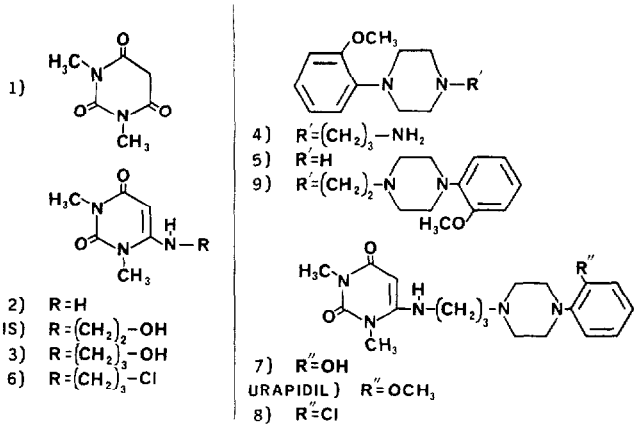

Fig. 1. Molecular structures of (1) and (4), urapidil equimolar hydrolysis products; (2), (3), (5) and (6), synthetic precursors; (7–9), isolated and identified impurities, IS, and urapidil.

was stored at −5°C and tested stable for six months. Table I gives the amounts of each SARM weighed into the same 100-ml volumetric.

Stock IS solution was prepared by weighing 15 mg IS into 100.0 ml HPLC eluent B (see below). The solution was stored at 5°C and prepared monthly. A 10.0-ml aliquot of stock IS was diluted daily to 100.0 ml in HPLC eluent A to prepare the working IS.

Reference solutions were prepared by adding 1.0, 0.5, and 0.2 ml of stock SARM mixture to 5.0 ml working IS, and 4 ml HPLC eluent A, then bringing to 10 ml with acetonitrile–water (9:1).

It was necessary that standard and sample solvent compositions be less than 20% in acetonitrile to avoid peak splitting of the polar analytes.

Spiked sample solutions

1.0, 0.8, 0.6, 0.4, and 0.2 ml of stock SARM mixture were added to 65 mg of urapidil fumarate SARM, 5.0 ml working IS was added along with 4 ml HPLC eluent A, then samples were brought to 10 ml with acetonitrile–water (9:1). A blank sample solvent was prepared without IS to determine the assay background.

TABLE I

STOCK SARM MIXTURE COMPOSITION

Standard Analytical Reference Materials weighed on a five-place milligram balance and combined in the same 100 ml volumetric.

	SARM								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
mg/100 ml	85	10	9	235*	95**	11	18	30	150

* As the trihydrochloride salt.
** As the dihydrochloride salt.

Samples

Urapidil fumarate test material (65 mg) was dissolved in 1 ml acetonitrile–water (9:1), 5.0 ml working IS solution, and 4 ml HPLC eluent A.

Apparatus

The analytical system comprised a Varian 5000 series liquid chromatograph equipped with a Rheodyne 7126 10-μl fixed-loop injector and Hewlett-Packard 1040A diode array detector set at 274 nm and 0.05 a.u.f.s. with the signal routed to a Beckman 450 controller for peak area integration. The flow-rate was 1.0 ml/min and the wavelength chosen was optimum for analyte sensitivity and minimal gradient background. Raw data was reduced off-line by the aforementioned computer programs on a Perkin Elmer 7500 series computer.

Columns and HPLC eluents

Columns utilized were a 250 × 4.6 mm I.D. analytical column and a 50 × 4.6 mm I.D. guard column, both packed with Spherisorb 5-μm Phenyl (Phase Separations).

Eluents consisted of: A, acetonitrile–water (1:200); B, acetonitrile–water (1:1), total volume of each 0.05 *M* in dibasic ammonium phosphate, and the pH of each adjusted with phosphoric acid to 4.8. The gradient program was started at sample injection, was linear from 100% A to 100% B over 40 min, recycled to 100% A in 4 min, and equilibrated 15 min.

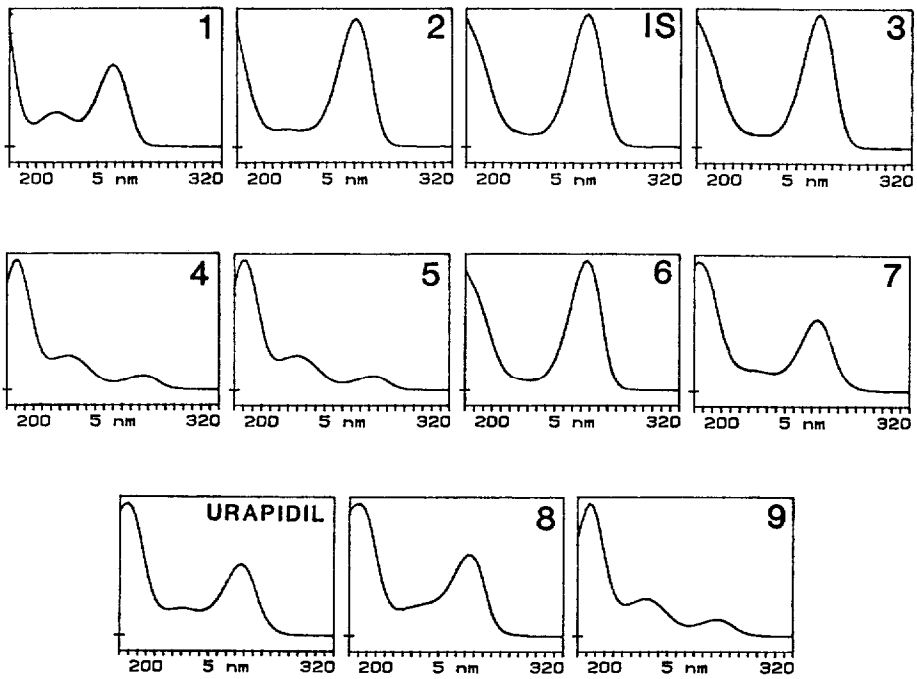

Fig. 2. HPLC-1040A UV spectral scans of SARMs 1–9, IS, and urapidil.

TABLE II

STATISTICAL RESULTS OF URAPIDIL FUMARATE SPIKED SAMPLE SOLUTIONS

n = 15 (3 days × 5 levels) for each analyte. Percent w/w expresses content in urapidil fumarate.

	SARM								
	1	*2*	*3*	*4**	*5**	*6*	*7*	*8***	*9*
Linearity***									
Slope	0.994	0.999	1.00	1.01	1.01	1.01	1.02	1.01	1.01
Intercept	3.43	0.04	−0.06	−6.29	−1.51	−0.54	−0.64	−0.35	0.96
r	0.9988	0.9996	0.9992	0.9994	0.9995	0.9992	0.9990	0.9997	0.9992
% Accuracy ($\bar{x}$)	100.6	100.0	100.5	100.3	100.4	99.9	100.9	101.0	101.4
± S.D.	3.1	2.1	3.1	2.3	2.6	2.6	3.1	2.2	2.7
Reproducibility [± % (w/w)]	0.019	0.001	0.002	0.027	0.011	0.002	0.004	0.003	0.033
Linear range tested [% (w/w)]	1.31–0.262	1.52–0.030	0.139–0.028	2.49–0.500	1.05–0.206	0.169–0.034	0.288–0.058	0.474–0.095	2.26–0.452
% (w/w)									
LOD	0.059	0.004	0.005	0.061	0.026	0.005	0.010	0.009	0.077
LOQ	0.108	0.007	0.009	0.138	0.056	0.011	0.021	0.019	0.149

* As the free base.

** By the method of standard addition with analyte 8 detected at ≈ 0.12 ppt.

*** µg added *vs.* µg found

RESULTS AND DISCUSSION

Fig. 2 shows the HPLC-1040A UV spectral scans of SARMs 1–9, IS, and urapidil. Comparing these at 274 nm and the respective molecular weights, indicated significant molar extinction differences, ruling out quantitation of these analytes by peak area normalization to urapidil.

Urapidil fumarate spiked sample solutions, reference solutions, and the blank were prepared and analyzed on three separate days. Fig. 3 shows representative chromatograms of these analyses along with the neat urapidil fumarate used for spiking. For the nine analytes combined, the mean accuracy was 100.6% ± 2.6% (n = 135 = 3 days × 5 levels × 9 analytes), with linearity of $y = 1.01x - 0.508$, $r = 0.9995$ (μg added *vs.* μg found), and reproducibility in terms of % (w/w) each analyte in urapidil fumarate was ±0.008 (mean) with a spread of ±0.001 to ±0.027. Table II

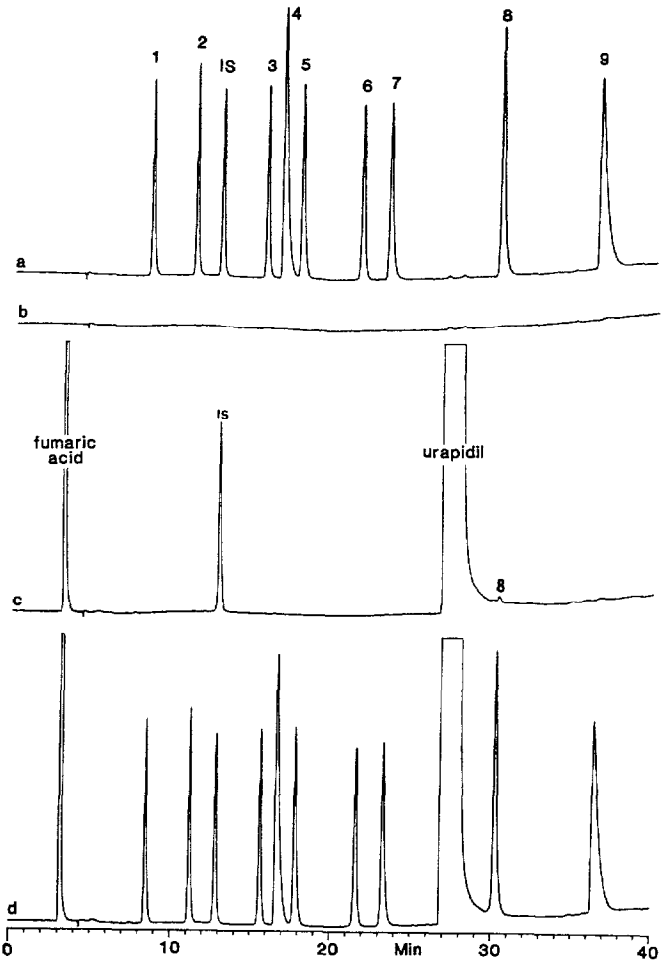

Fig. 3. (a) 1.0-ml reference solution. (b) Sample solvent blank. (c) Urapidil fumarate SARM lot used for spiking, with analyte 8 detected at ≈0.12 ppt. (d) 1.0-ml SARM solution spike.

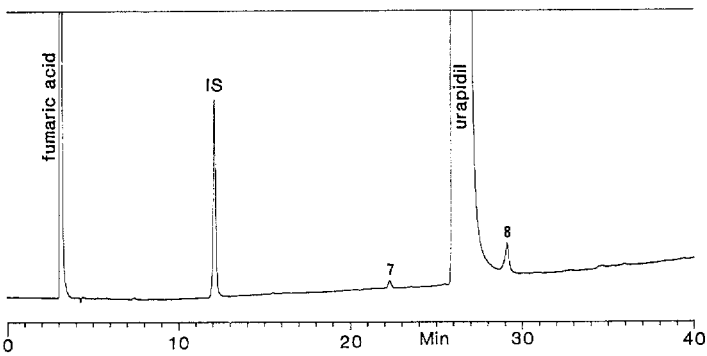

Fig. 4. Urapidil fumarate test sample. Analyte 7 detected at ≈ 0.013% (w/w) and 8 quantitated at 0.033% (w/w) (95% confidence). All other analytes, if present, are at less than their respective LODs. See Table II.

gives these results for each analyte independently and additionally the linear range tested with respective LODs and LOQs in terms of % (w/w). Fig. 4 shows a urapidil fumarate raw material lot analysis with quantitative results.

Chromatography

During optimization of the HPLC eluents it was found that raising the pH from 4.8 to 5.2 would reverse the elution order of analytes 4 and 5, giving the expected reversed-phase elution order. At pH 5.2 both the primary amine of 4 and the secondary amine of 5 are protonated; however, as the pH is lowered to 4.8 the piperazinyl nitrogen substituted with the propylamino group in 4 additionally protonates. This results in greater ionic character and hydrophilicity of 4 relative to 5 and can account for 4 eluting before 5. The analysis pH of 4.8 was chosen as it produced sharper peak shape of 4 yielding greater sensitivity.

The dibasic ammonium phosphate serves as an ion-suppressing reagent for analyte 1 (1,3-dimethylbarbituric acid) and as an amine solute modifier for the other components.

CONCLUSIONS

The described HPLC reversed-phase gradient analytical procedure is accurate, specific, and sensitive for evaluation of the synthetic purity and hydrolytic stability of urapidil fumarate.

The statistical method of Hubaux and Voss[9] for determining LODs and LOQs was found to be expeditious when compared to the previous procedure of analyzing low level samples to obtain detections at two times the signal-to-noise ratio. LODs determined at the 95% confidence level compared very well with values calculated at two times the signal-to-noise ratio.

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