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Roxatidine estimation in human fluids by UV derivative spectrophotometry ☆

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Abstract

Two simple and accurate methods for roxatidine assay in human serum and urine by derivative UV spectrophotometry are proposed. In serum the analyte is quantified after a solid-phase extraction procedure, whereas in urine it is determined after a selective extraction with 2-methylbutanol. In both methods the drug concentration is directly correlated with specific signals in the second and third derivative spectra of the sample extracts. Statistical analysis of the data from synthetic solutions confirms the good accuracy and precision of the methods.

Keywords: Roxatidine acetate HCl; Roxatidine acetate HCl metabolite; Serum; Urine; Solid-phase extraction; Ultraviolet derivative spectrophotometry

Roxatidine acetate HCl (I; 2-acetoxy-*N*-[3-[*m*-(piperidinylmethyl)phenoxy]propyl]acetamide hydrochloride) is a histamine H_2-receptor antagonist, used for the treatment of peptic ulcer disease (Scholtholt et al., 1988; Sewing et al., 1988).

I, after oral administration, is basified to II and then rapidly deacetylated to its major active metabolite roxatidine (III = Rox) (Collins and Pidgen, 1988; Rosch, 1988). The prodrug II is not detectable in biological fluids and all studies in the literature have been evaluated on III determination (Iwamura et al., 1985; Takabatake et al., 1985; Burrows et al., 1988). A third metabolite, identified as the amine derivative (IV), has been revealed in very low concentration (Honma et al., 1985).

For Rox determination in human biological fluids only a capillary gas chromatography method has been proposed (Burrows et al., 1988). In several pharmacokinetic studies, the drug and its major metabolites have been assayed by RIA (Hasegawa et al., 1984; Honma et al., 1985; Iwamura et al., 1985; Lameire et al., 1988).

Fig. 1 shows the absorbance spectra (H_2O) of the four products at the same concentration. The curves for II–IV almost coincided over the wavelength region selected for drug determination

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(250–300 nm). The high absorptivity of I has no influence due to its complete absence in the biological samples.

In this paper, all the procedures have been carried out on standard solutions of III (Rox), however, the results are valid for the mixture of all metabolites, since II and IV have the same absorptivity as III.

I was purchased from Sugiyama (Tokyo, Japan); II was obtained by extraction with diethyl ether from an aqueous solution of I to which $NaHCO_3$ had been added; Rox was prepared by stirring the salt I with aqueous 1 M NaOH (molar ratio 1:2) for 2 h at room temperature, and then extraction with diethyl ether; IV was obtained by stirring II with 1 M NaOH (molar ratio 1:4) at 100°C for 12 h and extraction with diethyl ether.

DL-2-Methyl-1-butanol was supplied by Aldrich Chemical. All the solvents were analytical grade.

Derivative spectra were recorded in 10 mm silica quartz cells using a Perkin-Elmer Lambda 15 spectrophotometer under the following instrumental conditions: scan speed, 2 nm s^{-1}; response (time constant), 1 s; spectral bandwidth, 1 nm; $\Delta\lambda$, 4 and 6 nm for second- and third-order derivative spectra, respectively.

Serum extracts: serum samples, collected from healty volunteers, were stored at −20°C and thawed to room temperature just before analysis. Laboratory solutions of Rox were prepared by adding to 1 ml of a pool of blank serum known amounts of an aqueous stock solution of Rox (40 $\mu g\ ml^{-1}$) to obtain solutions with Rox concentration ranging within 0.48–7.24 $\mu g\ ml^{-1}$.

0.5 ml of sample was added to acetonitrile (1 ml) and mixed in a 10 cm glass tube. The liquid phase was separated by centrifugation (3800 rpm for 20 min) and acetonitrile was evaporated at 60°C under a gentle stream of nitrogen. 0.5 ml of phosphate buffer pH 8 was added and the solution was applied to a CN Sep-Pak cartridge, previously activated by washing sequentially with acetone, ethanol and water. The cartridge was purged with 50 ml of water and then eluted with 2 ml of methanol-phosphate buffer (30:70) pH 8. The eluate was analysed against the solid-phase extraction solvent as a blank.

Urine extracts: blank human urine was collected from healty volunteers and stored at −20°C until analysis. Urine laboratory solutions were prepared by adding to 1 ml of a pool of blank urine amounts of Rox to obtain concentrations in the range 0.96–22.30 $\mu g\ ml^{-1}$.

1 ml of sample was added to 1 ml of 0.1 M KOH in a 10 cm glass tube. The solution was vortexed and then extracted twice with 1 ml of DL-2-methyl-1-butanol. The organic layers were separated by centrifugation (3800 rpm for 5 min)

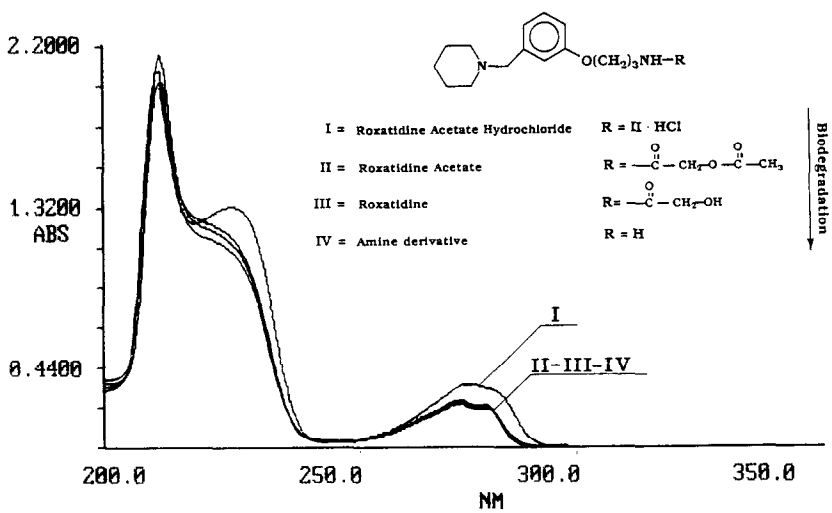

Fig. 1. Absorbance spectra of aqueous solutions of I–III and IV at a concentration of 19.34 $\mu g\ ml^{-1}$.

and transferred to a quartz cell. The solution was analysed against DL-2-methyl-1-butanol as a blank.

Analysis of serum: Fig. 2 shows the second-order derivative spectrum of blank serum and of its solutions containing increasing amounts of Rox, processed using the described method.

Because of the deproteinization and solid-phase extraction the blank serum shows a very low absorbance over the wavelength region 240–300 nm, allowing the detection of a specific signal whose amplitude value depends on the analyte concentration.

Liquid-solid extraction (LSE) was demonstrated to be better than liquid-liquid extraction (LLE). The major disadvantage of LLE when applied to serum samples is emulsion formation which causes loss of analyte.

Denaturing serum protein before the solid-phase extraction was demonstrated to facilitate rapid washing out of interfering compounds and to increase the amount of Rox extracted.

Alkalinization of the serum samples before extraction was found to increase the recovery of the drug, this being in the unionized form. For this reason the recovery of roxatidine under different pH conditions was investigated, demonstrating complete extraction recovery above pH 7.5.

Rox quantification was carried out correlating the peak-trough at 286–281 nm in the second derivative spectrum through the regression equation reported in Table 1.

Table 1
Regression equations for roxatidine evaluation (μg ml^{-1})

	Signal	Slope (±SD)	Intercept (±SD)	r
Serum	$^2D_{286,281}$	85.659 (±3.269)	0.384 (±0.152)	0.997
Urine	$^2D_{285,280}$	32.407 (±2.279)	−1.465 (±0.662)	0.995
	$^3D_{282}$	327.551 (±3.225)	−1.603 (±0.831)	0.997

Analysis of urine: Fig. 3 shows the second and third UV derivative spectra of methylbutanolic extracts of blank urine and of its solutions spiked with several Rox concentrations.

The drug exhibits a peak-trough at 285–280 nm in the second derivative spectrum and a maximum at 282 nm in the third derivative spectrum, which are independent of the matrix.

These signal amplitudes and the drug concentration were correlated through the regression equations listed in Table 1.

The analysis of a very high number of blank urine samples demonstrated that the contribution of the matrix had a negligible effect on the absorbance of the chosen signals, nevertheless, in rare cases, it can increase their amplitude values (5–10% in the examined samples).

This interference can be eliminated by correcting the signal value in the third derivative spectrum through a simple graphical procedure.

Fig. 4 shows the spectral curve of a blank urine extract which presents a high matrix contribution in the wavelength range of Rox absorbance and a second spectrum of the same sample spiked with a small amount of the analyte.

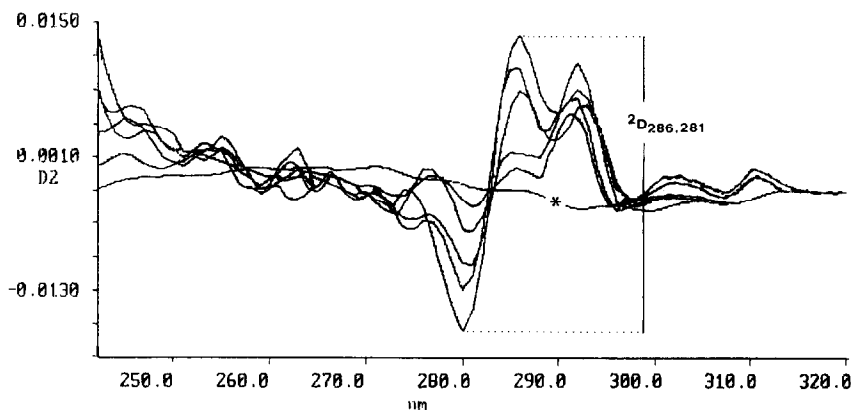

Fig. 2. Second derivative spectra of blank serum (*) and its solutions of Rox concentration ranging within 0.51–3.60 μg ml^{-1}.

Analyte concentration can be correlated with the amplitude A_{Rox} between the absorbance maximum and the joining line, resulting in isosbestic points 1 and 2. This amplitude A_{Rox} coincides geometrically with the Rox absorbance corrected for the matrix contribution (A_m).

A_m and A_{Rox} were calculated according to the following expressions:

$$A_m = \frac{(Y_2 - Y_1)(X_{282} - X_1)}{X_2 - X_1}$$

where Y and X represent the absorbance and the wavelength values, respectively.

$$A_{Rox} = Y_{282} - A_m$$

The good linearity of the calibration curves was confirmed by the high values of correlation coefficients (> 0.99).

Table 2 reports the analysis results of laboratory solutions in both fluids. The mean of the percent recovery values for serum was 99.83 with an RSD% of 3.01; for urine, recovery values ($\pm$RSD%) were 99.57 ($\pm$4.92) and 99.73 ($\pm$1.34) for the second- and third-order derivative, respectively.

The minimum detectable Rox concentration was determined to be 0.50 and 1.00 μg ml^{-1} for serum and urine, respectively.

The accuracy of the methods was estimated by performing a t-test on laboratory solutions pre-

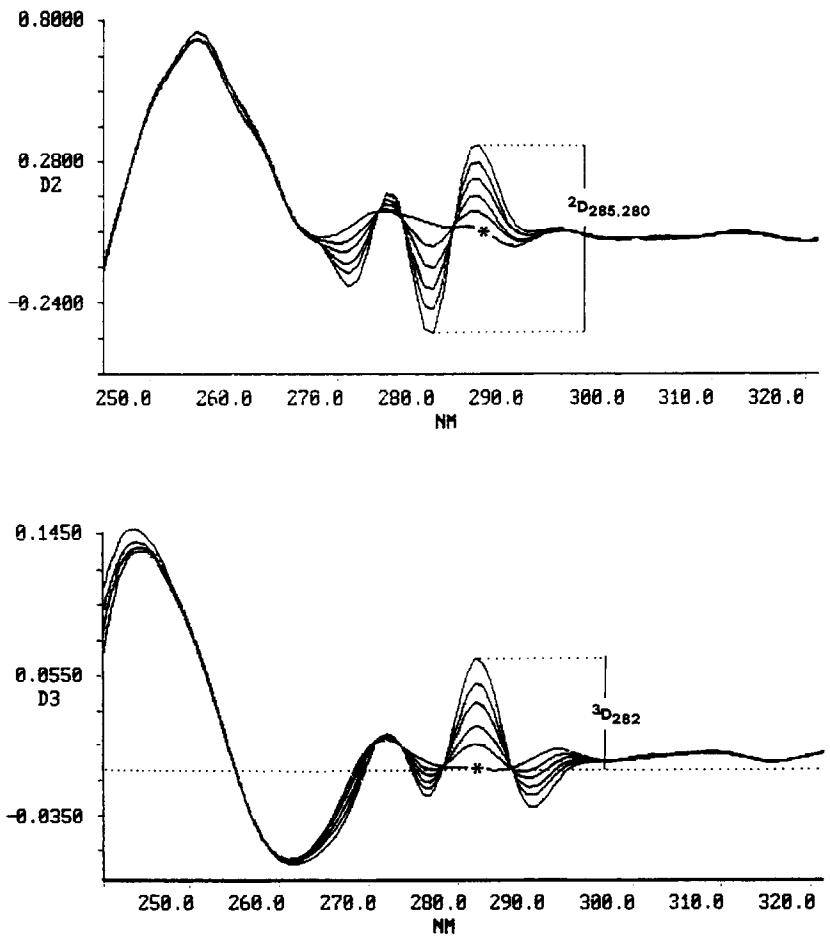

Fig. 3. Second and third derivative spectra of methylbutanolic extracts of blank urine (*) and its solutions with Rox concentration in the range 4.46–22.30 μg ml^{-1}.

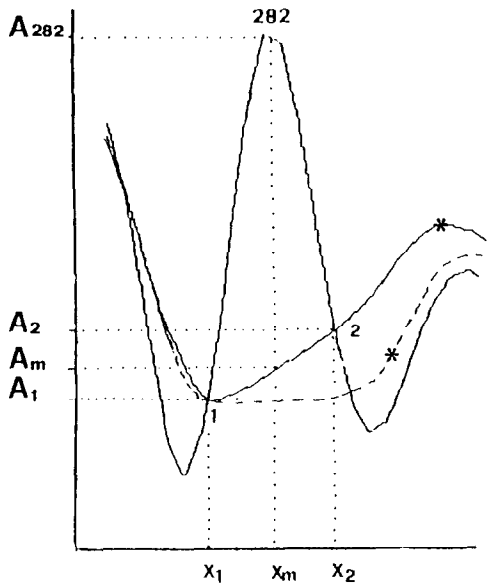

Fig. 4. Graphical procedure for matrix contribution correction in urine samples.

pared by adding a constant amount of Rox to the same blank matrix (3.76 and 13.38 μg ml^{-1} to serum and urine, respectively) in order to decide whether the difference between the true value and the experimental mean was significant. The calculated t values were 1.60 for serum and 1.91, 036 for urine, second and third derivative respectively, i.e., less than the critical value ($t = 2.78$; d.f. 4) at the significance level of 0.05, so the 'null hypothesis' is retained.

A paired t-test was also performed to compare the two derivative methods for urine analysis by using a variety of samples from different sources. Also in this case the observed value of t (1.46) was found to be less than the critical value (2.57) at the significance level of 0.05 (d.f. 5), hence the null hypothesis, that analytical methods are not subject to systematic errors, is followed.

For urine analysis the precision of the two methods was evaluated with a two-tailed F test. The calculated value ($F = 1.63$) was less than that tabulated ($F = 9.60$; $P = 0.05$; d.f. 4,4), therefore there is no significant difference between the two variances at this level.

Table 2
Recovery of roxatidine added (μg ml^{-1}) to serum and urine

Serum			Urine				
Added	2nd derivative		Added	2nd derivative		3rd derivative	
	Found	RSD%		Found	RSD%	Found	RSD%
0.88	0.89	2.30	1.04	1.12	3.67	1.01	0.94
1.62	1.56	3.45	4.46	4.46	5.66	4.48	0.23
3.76	3.90	5.45	8.92	8.23	4.62	8.99	2.10
4.16	4.02	2.63	13.38	13.23	6.34	13.43	1.04
5.35	5.27	2.57	17.84	17.82	5.93	17.78	1.12
6.27	6.44	1.68	22.30	22.01	3.31	22.31	2.61

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References

Burrows, J., Jolley, K. and Sullivan D., Determination of roxatidine in human plasma, urine and milk by capillary gas chromatography using nitrogen selective detection. *J. Chromatogr.*, 432 (1988) 199–208.

Collins, J. and Pidgen, A., Pharmacokinetics of roxatidine in healthy volunteers. *Drugs*, 35 (Suppl. 3) (1988) 114–118.

Hasegawa, Y., Osawa, H. and Mine T., Phase I study of TZU-0460, a novel histamine H_2-receptor antagonist: 2. 7 and 56 consecutive day study. *Jap. Pharmacol. Ther.*, 12 (1984) 649–652.

Honma, S., Akutsu, R., Iwamura, S., Kawabe, Y. and Tsukamoto, K., Metabolic fate of 2-acetoxy-*N*-[3-[*m*-(1-piperidinylmethyl)phenoxy]propyl]acetamide hydrochloride (TZU-0460), a new H2-receptor antagonist: 4. The metabolism in man. *Oyo Yakuri*, 30 (1985) 553–563.

Iwamura, S., Akutsu, R., Honma, S. and Tsukamoto, K., Metabolic fate of 2-acetoxy-*N*-[3-[*m*-(1-piperidinylmethyl)phenoxy]propyl]acetamide hydrochloride (TZU-0460), a new H2-receptor antagonist: 1. Blood concentration, excretion and tissue distribution in rats during and after consecutive oral administration. *Pharmacometrics*, 30 (1985) 321–328.

Lameire, N., Rosenkrantz, B., Maass, L. and Brockmaier, D., A pharmacokinetic study of roxatidine acetate in chronic renal failure. *Drugs*, 35 (Suppl. 3) (1988) 48–55.

Rosch, W., A comparison of roxatidine acetate 150 mg once daily and 175 mg twice daily in gastric ulcer healing. *Drugs*, 35 (Suppl. 3) (1988) 127–133.

Scholtholt, J., Bickel, M. and Herling, A., A review of the animal pharmacology of roxatidine acetate. *Drugs*, 35 (Suppl. 3) (1988) 30–37.

Sewing, K., Beil, W. and Hannemann, H., Comparative pharmacology of histamine H_2-receptor antagonists. *Drugs*, 35 (Suppl. 3) (1988) 25–29.

Takabatake, T., Ohta, H., Yamamoto, Y., Ishida, Y., Hara, H., Nakamura, S., Ushiogi, Y., Satoh, S. and Hattori, Y., Pharmacokinetics of TZU-0460, a new H_2-receptor antagonist, in patients with impaired renal functions. *Eur. J. Clinic. Pharmacol.*, 30 (1985) 709–712.