HPLC method for determination of 7-Theophyllinylacetyloxyglycol in rabbit serum

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ABSTRACT

A convenient, rapid and sensitive method for determination of ester derivative of 7-theophyllineacetic acid with propylene glycol in rabbit serum was developed. The procedure involved liquid-liquid extraction of the compound from matrix with chloroform:isopropanol (1:1), followed by RP-HPLC using LiChrosorb RP-8, 250 mm x 4.6 mm, 5 μm column and UV detection at 274 nm. The elution was achieved isocratically with a mobile phase consisted of methanol:water:tetrahydrofuran:o-phosphoric acid (42:55:2.5:0.5 v/v/v/v). 8-Bromotheophylline was applied as suitable internal standard. The assay was validated and found to be accurate, precise and specific. The calibration curve was linear over the concentration range of 5 to 50 μg/ml with r^2 values greater than 0.99. The reported method was successfully employed for a preliminary pharmacokinetic study of the ester in rabbits. The two-compartmental pharmacokinetic model was used to fit the ester serum concentration-time curve. 7-Theophyllinylacetyloxyglycol was rapidly absorbed after oral administration. Maximum serum concentration was 35.60 ± 3.01 μg/ml that occurred at 2 h post-dosing.

Key words: 7-Theophyllinylacetyloxyglycol, liquid chromatography, validation, rabbits, pharmacokinetics
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INTRODUCTION

7-Theophyllineacetic acid (1,2,3,6-tetrahydro-1,3-dimethyl-2,6-dioxopurine-7-acetic acid) is a xanthine derivative extensively used for its diuretic, cardiac stimulant, and smooth muscle relaxant pharmacological effects. Several studies reported in the literature [1-4], indicated that the compound is poorly absorbed after intravenous and oral administration. An appropriate approach to improve the absorption of the drugs as well as to reduce toxicity and side effects would be alteration of their physicochemical characteristics by chemical modification [5,6]. 7-Theophyllineacetic acid contains a carboxylic function and an obvious approach for chemical derivatization is to prepare ester and amide derivatives [7-11].

Four esters of 7- Theophyllineacetic acid with several glycols have been recently synthesized [12, 13] as potential bronchodilating agents and their chemical and *in vitro* enzymatic stability have been evaluated [14-19]. The results derived indicated that the investigated esters are quite resistant to chemical and enzymatic degradation.

The objective of this paper was to develop and validate a simple and rapid HPLC procedure suitable for determination of one of synthesized esters-1-(7-theophyllinylacetyloxy)-3-hydroxypropane (Fig. 1) in rabbit serum after oral administration in rabbits.

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$$H_3C-N \quad CH_2COOCH_2CH_2CH_2OH$$
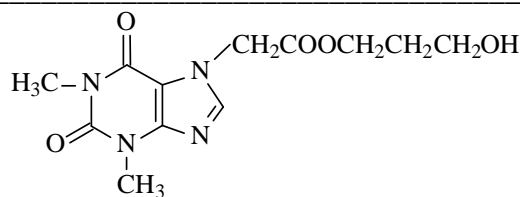
Fig. No 1. Chemical structure of 1-(7-theophyllinylacetyloxy)-3-hydroxypropane

MATERIALS AND METHODS

Chemicals and reagents
HPLC methanol and tetrahydrofuran were used to prepare the mobile phase. All other reagents were of analytical grade. 1-(7-theophyllinylacetyloxy)-3-hydroxypropane was kindly supplied from Zlatkov et al. [12]. 7-Theophyllineacetic acid and 8-bromotheophylline were supplied from Sigma-Aldrich (Germany).

Experimental animals
Male New Zealand rabbits (mean body weight 2.46±0.75 kg) were kept under standard laboratory conditions (20ºC, humidity 60 %, cycle – 12 hours light, 12 hours dark), with unrestricted access to granulated standard food and water. The trial was performed in compliance with the requirements of European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS123, 1991).

Instrumentation and chromatographic conditions
The HPLC system consisted of a Shimadzu LC-10A system equipped with a LC-10 AS pump, SPD-10A variable wavelength detector, SIL-10A autosampler and SCL-10A system controller. The mobile phase of methanol:water:tetrahydrofuran:o-phosphoric acid (42:55:2.5:0.5 *v/v/v/v*) was delivered at a flow rate of 1 ml/min. Chromatographic column LiChrosorb RP-8, 250 mm x 4.6 mm, 5 μm (Merck, Germany), equipped with additional guard column (20 x 4 mm) was used. The eluate was monitored using UV detector with wavelength at 274 nm.

Method validation
Extraction procedure
To 0.2 ml of serum 2.0 ml mixture of isopropanol-chloroform (1:1) containing 8 μg/ml 8-bromtheophylline was added. After shaking for 1 min on a Vortex mixer, the mixture was centrifuged at 3000 rpm for 3 min. A 1.0 ml aliquot of the clear organic solvent was removed and then evaporated to dryness at 65-70°C. The residue was dissolved in 0.5 ml of mobile phase and 20 μl aliquots were injected into the chromatographic system.

Linearity
Calibration curves were constructed for ester in mobile phase as well as in serum. Stock solutions of ester and internal standard were prepared in mobile phase. Blank serum was obtained by centrifuging blood samples at 5000 rpm for 5 min after which the supernatant was collected and stored at –20°C. After thawing the serum was spiked with appropriate amounts of stock solution to yield final concentration of the ester in the range 5 to 50 μg/ml.

Accuracy
The accuracy of the method was verified by analysis of model mixtures obtained by adding known amounts of ester to blank serum. The concentrations of the mixtures were 15, 25 and 30 μg/ml. Three replicates were performed for each concentration studied. The extraction recovery of ester from biological matrix was calculated by comparing concentrations measured in the serum with the concentrations added.

Precision
The precision of the method was assessed by repeated analysis of serum specimens containing known concentration of the compound (30 μg/ml).

Application to pharmacokinetic study
The method proposed was applied to determine the ester concentrations in serum. Four healthy New Zealand male rabbits were utilized. The ester was administrated in dose 50 mg/kg b.w. Blood samples (0.5 ml) were collected at 0.25, 0.5, 1, 2, 3, 4, 6, 12 and 24 h after application of the compound from the ear marginal vein. Forty minutes after

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withdrawal blood was centrifuged for 5 minutes at 10000 rpm to obtain serum. The serum was frozen at -20ºC until analysis. Pharmacokinetic parameters were obtained for each subject using a computer program PK Solutions 2.0.

RESULTS AND DISCUSSION

Under the described chromatographic conditions peaks of serum components, internal standard (t_r = 5.46 min) and ester (t_r = 9.07 min) were well resolved. No interfering peaks were observed in chromatogram of blank serum at the retention times of investigated compounds, which verify the specificity of analytical method. The chromatogram of the serum sample obtained 6 h after oral administration of ester is shown in Fig. 2.
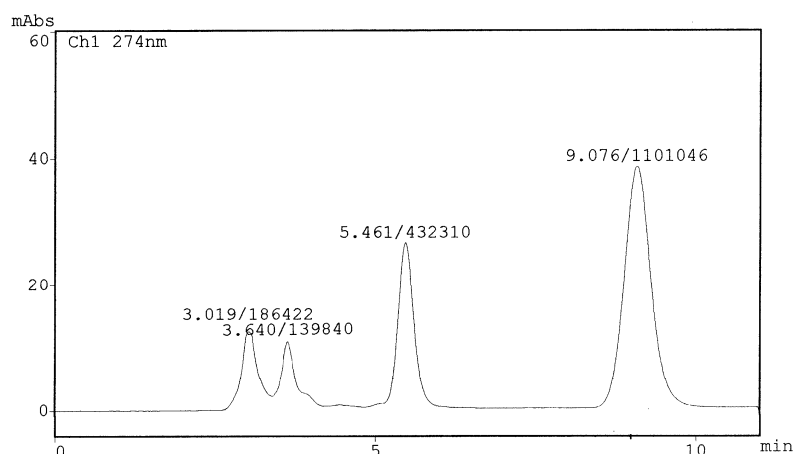
Fig. No 2. Chromatogram obtained from analysis of serum sample taken 6 h after administration of ester

Besides the serum constituents, ester and internal standard, a peak of hydrolysis product –7-theophyllineacetic acid (t_r = 3.64 min) can be seen also. The concentrations determined were 4.31 μg/ml for the acid and 9.17 μg/ml for the ester, respectively.

Validation data
Peak area ratios of ester to internal standard showed a linear relationship to serum concentration within the range 5 to 50 μg/ml. The quantification and detection limits for ester were 40 ng and 20 ng, respectively. The good linearity of the calibration curves was confirmed by the high value of correlation coefficients (r^2=0.9998). Data concerning validation procedure were shown in Table 1. The average extraction recovery of examined ester from biological matrix was 96.78 %. The relative standard deviation (RSD) obtained in study of precision was below 2 %.

Table No 1. Accuracy and precision of the method

Precision		Accuracy		
Taken amount, μg/ml	Found amount, μg/ml	Taken amount, μg/ml	Found amount, μg/ml	Recovery, %
30.00	30.06	15	15.38	102.6
	29.92		14.76	98.46
	29.30		15.08	100.5
	29.45	25	22.77	91.08
	29.82		23.85	95.40
	29.39		23.18	92.72
		30	29.23	97.44
			28.62	95.38
Mean: 29.66			29.23	97.44
Sd=0.316		Mean: 96.78		
RSD (%) =1.07		Sd=3.610		
		RSD (%) =3.73		

Pharmacokinetic evaluations
The ester was rapidly absorbed and highest serum concentration 35.60 +/- 3.01 μg/ml was reached at the second hour after administration. The serum levels of the ester were found to follow two-compartmental model. The pharmacokinetic parameters obtained were presented in Table 2.

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Table No 2. Pharmacokinetic parameters of ester

Subject	Pharmacokinetic parameters					
	C_{max} (μg/ml)	k_a (h^{-1})	α (h^{-1})	K_e (h^{-1})	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (μg/h/ml)
Rabbit I	36.81	1.37	0.83	0.023	29.92	392.2
Rabbit II	32.63	1.54	0.71	0.015	46.52	440.4
Rabbit III	39.41	1.43	0.76	0.024	28.75	421.2
Rabbit IV	33.85	2.00	0.79	0.030	23.00	448.3

CONCLUSION

A sensitive, precise and accurate HPLC method for the determination of 1-(7-theophyllinylacetyloxy)-3-hydroxypropane in small volume of biological fluid was described. The elaborated analytical procedure was successfully applied to pharmacokinetic study of ester in rabbits.

REFERENCES

[1]   S. Sved, J. McGilveray, N. Beaudoin, *Biopharm Drug Dispos.,* **1981**, 2, 177.
[2]   J. Ufkes, R. Leeuwen, M. Ottenhof, A. Zeegres, J. Ziudema, *Arch Int Pharmacodyn Ther.,* **1981**, 253, 301.
[3]   J. Owen, K. Nakatsu, *Biopharm Drug Dispos.*, **1980**, 1, 179.
[4]   J. Fleetham, J. Owen, B. May, P. Munt, K. Nakatsu, *Thorax*, **1979**, 34, 540.
[5]   A. Rasheed, I. Theja, C.K. Ashok Kumar, Y. Lavanya, P. Pavindra Royal, S. Vamsee Krishna, *Der Pharma Chemica*, **2009**, 1, 59.
[6]   S. Dhaneshwar, R. Buchade, S. Vetal, H. Gautam, M. K. Tewari, H. Karandikar, *Der Pharma Chemica*, **2011**, 3, 354.
[7]   G. Momekov, I. Pencheva, B. Tsvetkova, P. Peikov, *Compt. Rend. Acad. Bulg. Sci.*, **2010**, 63, 1295.
[8]   A. Zlatkov, P. Peikov, G. Momekov, I. Pencheva, B. Tsvetkova, *Der Pharma Chemica*, **2010**, 2, 197.
[9]   G. Danila, M. Ungureanu, M. Pavelescu, G. Rusu, I. Sandu, *Rev. Med. Chir. Soc. Med. Nat. Iasi*, **1990**, 94, 581.
[10] M. Eckstein, M. Cegla, *Acta Pol. Pharm*, **1987**, 44, 12.
[11] R. Czarnecki, T. Librowski, M. Pawlowski, *Pol. J. Pharmacol.*, **2001**, 53, 131.
[12] A. Zlatkov, Peikov, P., Danchev, N., Ivanov, D., Tsvetkova, B., *Arch. Pharm.*, **1998**, 331, 313.
[13] B. Tsvetkova, J. Tencheva, P. Peikov, *Acta Pharm.*, **2006**, 56, 251.
[14] T. Rajesh, K.S. Lakshmi, S. Sharma, Der Pharma Chemica, 2009, 1, 12.
[15] B. Tsvetkova, J. Tencheva, P. Peikov, *Acta Pharm.*, **2001**, 51, 317.
[16] A. Patni, T. Monif, A. Khuroo, A. Tiwary, *Der Pharmacia Lettre*, **2010**, 2, 41.
[17] D. Reddy, A. Bapuji, V. Rao, V. Himabindu, D. Raju, S. Syedba, S. Ravinder, H. Ravikiran, *Der Pharmacia Lettre*, **2011**, 3, 54.
[18] B. Tsvetkova, J. Tencheva, P. Peikov, *J. Food and Drug Anal.*, **2003**, 11, 191.
[19] B. Tsvetkova, J. Tencheva, P. Peikov, *Pharmacia*, **2005**, LII, 30.