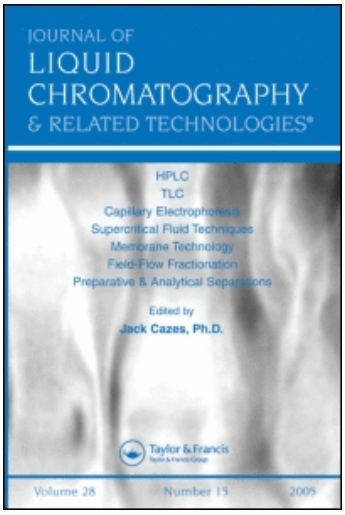

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APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN THE DETERMINATION OF TAUROMUSTINE IN PRESENCE OF ITS METABOLITES AND DEGRADATION PRODUCTS

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ABSTRACT: A reversed phase high performance liquid chromatography (HPLC) method was developed for the determination of tauromustine (TM) in presence of its metabolited in biological fluids and its degradation products in tablets. The proposed HPLC method was conducted using a lichrosorb C_{18} column [250 X 4 mm], with acetonitrile - water - acetate buffer pH5 (40 : 55 : 5) eluent, the detection was affected at 235 nm. The detector response was linear in the range 0.2-4 μg/ml for TM with minimum detectability (S/N=2) of 2 ng/ml. The proposed HPLC method was applied to the determination of TM in presence of its metabolites in biological fluids. The percentage recoveries of TM and its metabolites from spiked urine range from 93.1 to 92.9 and for plasma 90.3 to 89.6 . The proposed HPLC method was used to study the kinetics of degradation of TM in standard solution as a function of temperature and alkalinity. TM degraded followed first-order kineties in agreement with the Arrhenius theory. The proposed

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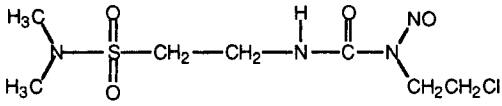
HPLC method was applied in tablets and the results obtained were in good agreement with those obtained by the reference method.

Introduction



Scheme I

Tauromustine,1-(2-chloroethyl)-3-[2-(dimethylamino-sulphonyl)ethyl] 1-nitrosourea (TM) (scheme I), is a novel antitumour agent based on the endogenous aminoethane sulphonic acid, taurine. Preclinical studies with tauromustine have revealed its potent antitumour activity against several experimental tumours in vivo and in vitro [1,2] and its efficacy against nitrosourea - resistant tumours [3].

An HPLC method was described for the determination of tauromustine and its metabolites in plasma and urine [4,5]. However, this method used a cyano column, the half life of which is short [6], as it efficiency decrease with time, necessitating frequent changes of the column. Recently, a polarographic method was developed for the determination of tauromustine in tablets (7) and a fluorimetric method was described for the determination of tauromustine in formulations and biological fluids (8).

Although, tauromustine is easily decomposed in the dry state, nothing was published concerning its determination in presence of its degradation products. It is thus evident that, there is still a need for a more reliable method for the determination of tauromustine in presence of its degradation products as well as its metabolites.

Experimental

Apparatus:

HPLC pump : LKB 2150 provided by LKB-produkter AB, S-16126 BROMMA, Sweden.

Column : 5 µm Lichrosorb RP 18 (250 X 4 mm)
Injector loop : 50 µL.
Detector : LKB 2151 variable wavelength monitor.
Recording integrator : LKB 2220
Filter : Gamma 16 (5 µm)

Reagents and Materials:

Tauromustine, tablets and metabolites were kindly provided by Pharmacia, LEO, AB, Helsingborg, Sweden. Stock Solutions; aqueous solutions containing 1 mg/ml of TM was prepared, this solution was further diluted with water to give the final concentration required for preparation of calibration graph.

The mobile phase was an isocratic mixture of acetonitrile - water - acetate buffer (9) pH 5 (40 : 55 : 5), degassed befor use.

HPLC Analysis of Standared Solution of TM.

Standared solution containing various concentration of TM were chromatographed and the response peak heights were measured. The concentration of TM was calculated from the calibration graph or from the regression equation.

HPLC Analysis of TM Tablets:

Twenty tablets (20 and 50 mg) were weighed and pulverized. An accurately weighed amount of the powder equivalent to 100 mg of TM was extracted with 5X15 ml water, filtered and completed to 100 ml with water. Working solutions were prepared by dilution with water to contain 0.2 - 4 µg/ml.

HPLC Analysis of TM and its Metabolites in Biological Fluids:

The plasma or urin samples (5ml) were acidified with 2 M hydrochloric acid [10 µl per ml of plasma or urin], aqueous solutions of 1mg/ml of tauromustine (TM), demethyl tauromustine (DMTM) and didemethyl tauromustine (DDMTM) were added and the solution was thoroughly mixed in a vortex mixer for 15 min and diluted to 25 ml in a measuring flask with phosphate buffer (pH 6). The mixture was transformed to 50 ml separating funnel and extracted with 3X10 ml of chloroform, the organic phase was filtered through dry sodium sulphate then evaporated under a gentle stream of nitrogen. The residue was dissolved in bidistilled water. The solutions were then diluted with water to the working calibration region.

Effect of heat on degradation rate of TM in raw material.

TM standard solution was stored at ambient temperature (25± 0.2^{0}C) and 50 ^{0}C. Zero-time sample measurements were carried out

when the study began only on samples from flasks to be stored under ambient conditions. All original flaskes samples stored under accelerated conditions. The flasks were re-closed tightly by hand between sampling. For all storage conditions, the entire study was performed on duplicate flaskes of TM raw material. For aqueous solutions, samples equivalent to 100 mg of TM raw material were dissolved in 100 ml water, samples were diluted 1: 100 using water and poured into 10 ml flaskes and closed. the flaskes were placed in oven set at 50 ^{0}C and at specified time intervals individual samples were taken for analysis.

The percentage recoveries of TM in the pharmaceutical preparations were calculated either from a calibration graph obtained under the same conditions or by the external standard method.

Results and Discussion

The effect of acetonitrile content and pH on the number of theoretical plate (NTP) and phase capacity ratio (K`) for TM were studied. Adjusting the pH of 40% acetonitrile to 5 by using acetate buffer gave high resolution power of the mobile phase.

The resulting peak heights were proportional to TM concentration over the range 0.2-4 μg/ml with minimum detectability [S/N=2] of 2 ng/ml. Linear regression analysis of the plot of TM concentration over the cited concentration range and the measured peak heights gave the following regression equation:

$$C = 0.0107 \pm 0.567\ P \qquad (R^2 = 0.9997)$$

Where C is the concentration of TM in μg/ml and P (cm) is the peak height, R is the correlation coefficient.

To test the validity of the method it was applied to the determination of standard solutions of TM. The results abridged in Table I show that the proposed method is accurate and precise. The proposed method was further applied to the determination of TM in tablets, the results are in good agreement with reference method according to Table I . Tablets excipients such as talc, starch, lactose, gum, magnesium stearate did not interfere with the assay.

A typical chromatogram of TM in presence of its metabolites DMTM and DDMTM after extraction from biological fluids are shown in Fig. 1. The retention time was suitable enough to allow separation

Table I : Performance data for the determination* of tauromustine

Determination of tauromustine in	$\bar{X}$	S . D.	t**	F**
(1) Raw material by : -				
a - Proposd mthod .	100.1	0.7	0.1 (2.1)	1.1 (5.6)
b - Reference method (8)	100.1	0.6		
(2) Tablet (20 mg) by : -				
a - Proposed method .	99.8	0.7	1.1 (2.1)	1.5 (5.6)
b - Reference method (8)	99.6	0.6		
(3) Tablet (50 mg) by : -				
a - Proposed method .	99.9	0.6	1.1 (2.1)	1.6 (5.6)
b - Reference method (8)	99.3	0.7		
(4) In plasma	89.9	2.0		
with				
a - D M T M	89.6	0.6		
b - D D M T M	90.3	0.7		
(5) In urine	92.9	2.7		
with				
a - D M T M	93.3	1.5		
b - D D M T M	93.1	1.3		

* Each result is the average of four separate determination .
** Values in brackets are tabulatd -t- and -F- values (P = 0.05)

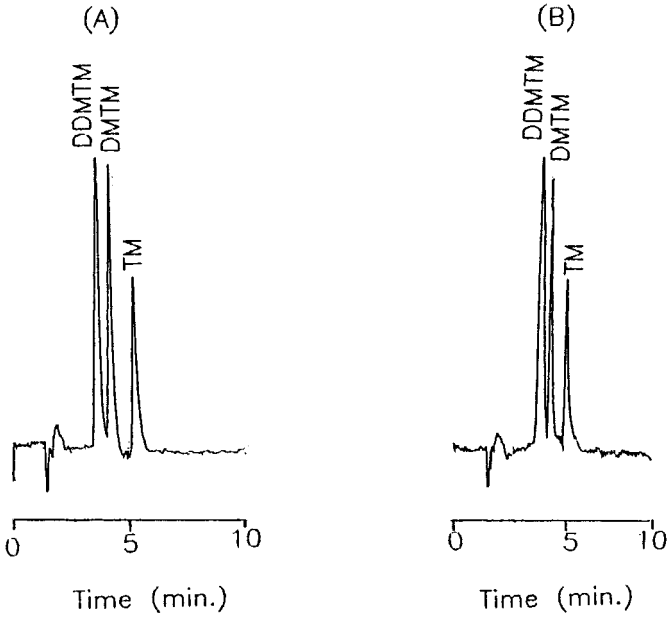


Fig. 1 :Chromatograms of urine (A) and plasma(B) spiked with 2.0 µg/ml DDMTM,DMTM and TM,the mobile phase; acetonitrile-water-acetate buffer (40:55:5) pH 4.5 for plasma and pH 6 for urine, flow rate 1.5 ml/min,detector wavelength 235 nm.

of TM from its metabolites. The method was tested for the presence of interfering peaks originating from various sources. These sources of interference could be eliminated by adjusting the pH of the mobil phase. Normally a pH value of 4.5 was chosen for plasma samples and of 6 for urine samples. The plasma or urine samples were acidified first then adjusted to pH 6 before extraction with organic solvent, this procedure minimized interference from other endogenous substance. Samples were extracted according to the procedure described above and the results are show in Tables I.

Accelerated stability study on TM was performed by heating the aqueous solutions of TM at 50 ^{0}C for different periods of time ranging from 5 - 120 min. The degraded solutions were analysed by the proposed method and the percentage remaining of undecomposed drug were calculated from the corresponding

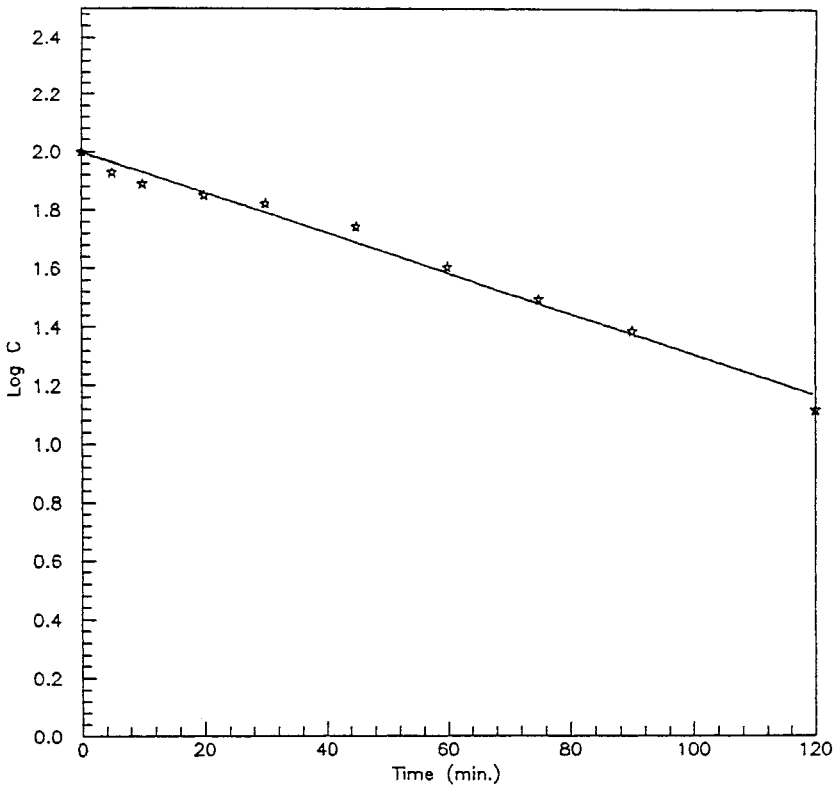


Fig. 2 : Plot of Log concentration remaining versus pH for the degradation of TM in aqueous solution at 50 C.

calibration graph . Plotting the value of the concentration remaining [Log. C.] against time indicate that TM degradation followed first-order kineties and gave a linear relationship [Fig.2.]. The presence of the degradation products of TM did not interfere in the determination of TM. Similarly, the stability of TM in alkalin medium (0.1 M sodium hydroxide) was studied. The drug was completely degraded in alkaline medium at once and could not be detected by proposed HPLC method.

On conclusion, the proposed HPLC method is rapid, sensitive, selective and accurate. The method is suitable for regular determination of TM. It is suitable for checking stability of its formulations.

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