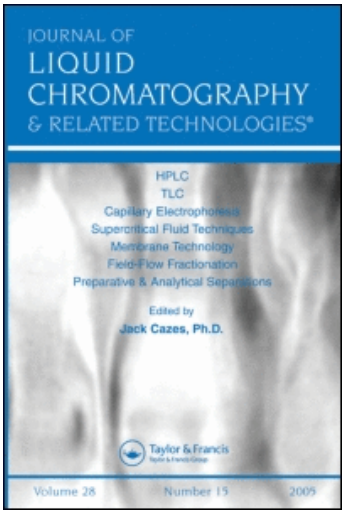

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DETERMINATION OF TRANYLCYPROMINE IN URINE AND PHARMACEUTICAL FORMULATION BY HPLC USING SYMMETRY COLUMN

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ABSTRACT

A simple isocratic reversed phase HPLC method for the determination of tranylcypromine sulfate (TCP) is described. The column used is Symmetry C_{18} (Waters Corp., Milford, MA, USA) and the mobile phase consists of 50 mM KH_2PO_4 (pH 4.55):methanol:water (20:10:70). Tranylcypromine sulfate is monitored by U.V. detection at 264 nm. The calibration curve of tranylcypromine sulfate is constructed over the range 25 nmol - 375 nmol/mL with a correlation coefficient 0.999. The method is specific and sensitive with a lower limit of detection 5 nmol/mL, the intra-assay and inter-assay relative standard deviation were below 10%. The validated assay procedure was applied to determine the tranylcypromine sulfate in urine and also to evaluate TCP in pharmaceutical preparation. Detailed methodology and the advantage of this C_{18} reversed phase is presented.

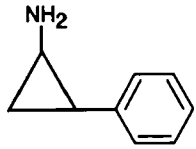

Figure 1: The chemical structure of tranylcypromine (TCP).

INTRODUCTION

Tranylcypromine (TCP) is one of many psychotropic agents which is administered as racemate, chemically known as (±) trans-2-phenylcyclopropylamine (Figure 1). Tranylcypromine is a monoamine oxidase (MAO) inhibitor. It is used as an antidepressant drug, particularly for atypical depression and for the treatment of phobias.[1] It was withdrawn from the market because of severe side effect related to MAO activity such as hypertensive episodes and cerebrovascular accident.[1] In 1964, it was reinstated for limited use.[2]

Baldessarini[1] reported that the antidepressant action of TCP is generally assumed but not definitely shown to be due to MAO inhibition. The same report stated that the maximal oxidase inhibition is produced in few days, whereas, the antidepressant effect takes two to three weeks to develop.[1] It was also proposed that catecholamine uptake inhibition as a possible mechanism for antidepressant effect of TCP.[3]

Furthermore, it is reported that chronic but not acute treatment with low doses of TCP increases extracellular 5-hydroxytryptamine (5-HT) concentration, suggesting that clinical effect of this MAO inhibitor are related to its capacity to enhance serotenergic transmission.[4]

It may be essential to monitor TCP concentration in biological fluids. Herein we describe a simple reliable and rapid HPLC method for the determination of TCP sulfate in biological fluid and pharmaceutical preparation.

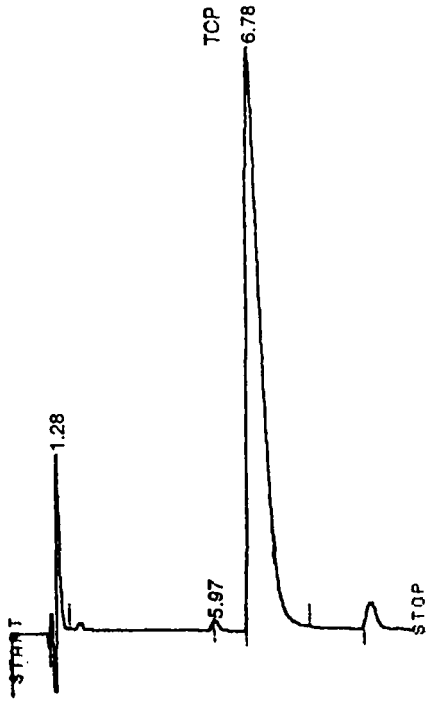

Figure 2: Chromatogram of tranylcypromine. Column:Symmetry C_{18} (150 mm x 3.9 mm I.D., particle size 5 μm); mobile phase; 50 mM KH_2PO_4 (pH 4.55):methanol:water (20:10:70); flow rate: 1 mL/min; chart speed: 0.5 cm/min; temp 23°C; detector UV 264 nm; sensitivity 0.01 aufs; sample quantity: 1 nmol.

MATERIALS AND METHODS

Chromatography

The HPLC system consisted of a Bio-Rad 1350 Solvent Delivery Pump, a Rheodyne Model 7125 Injector, a Waters Lambda Max 481 variable wavelength detector set up at 264 nm and Hewlett-Packard 3394A integrator. The columns used was Symmetry C_{18} reversed phase (150 mm x 3.9 mm i.d. particle size 5 μm) Lot No. T50961 kindly supplied by Dr. Michael E. Swartz, Waters Corp., Milford, MA, USA and Nucleosil C_{18} reversed phase (250 mm x

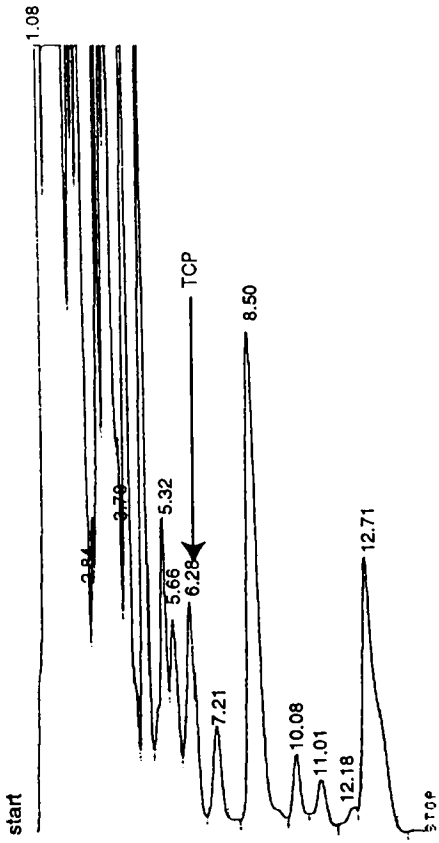

Figure 3: Chromatogram of spiked urine extract. Column:Symmetry C_{18} (150 mm x 3.9 mm I.D., particle size 5 μm); mobile phase; 50 mM KH_2PO_4 (pH 4.55):methanol:water (20:10:70); flow rate: 1 mL/min; chart speed: 0.5 cm/min; temp 23°C; detector UV 264 nm; sensitivity 0.01 aufs.

4.6 i.d., particle size 5 μm), Lot. No. 102H3409 purchased from Sigma-Aldrich, St. Louis, MO, USA. The mobile phase consists of 50 mM KH_2PO_4 (pH 4.55):methanol:water (20:10:70). All other chromatographic conditions are described in figure legends.

Chemicals

Authentic tranylcypromine sulfate, control no. 11605 kindly supplied by Dr. Ober from Rohm Pharma (Darmstadt, Germany). ACS-grade n-octanol, sodium hydroxide, HPLC-grade methanol, potassium phosphate and phosphoric acid were purchased from Fisher Scientific (Fairlawn, NJ, USA). Boric acid was obtained from BDH Chemicals, Ltd., Poole, England.

Sample Preparation and Extraction

Authentic TCP sulfate solution is dissolved in the mobile phase. Urine sample spiked with TCP sulfate and tablet were extracted according to Krugers Dagneaux et al.,[5] with some minor modification.[6]

Following the addition of 1 mL of 0.2 M boric acid/KCl buffer to 1 mL of urine the final solution was brought to pH 8.5 in a glass tube, the contents were extracted with 5 mL of a mixture of 10% n-octanol and 90% n-hexane. The tubes were repeatedly inverted for 2 min and centrifuged at 1500 X g for 15 mins. The organic layer was recovered and further extracted with 4 mL of n-octanol and 1 mL of 50 mM H_3PO_4, in the same manner. Following centrifugation, the organic layer was discarded and 20 μL of aqueous portion was injected onto the HPLC system.

RESULTS AND DISCUSSION

Chromatograms

A typical chromatogram of TCP sulfate is presented in Figure 2, while the chromatogram of spiked urine extract is shown in Figure 3. Figure 4 shows chromatogram of TCP sulfate on Nucleosil C_{18} column under the same chromatographic condition.

Linearity

The calibration curve of the TCP sulfate was constructed over the range 25 nmol-375 nmol/mL with a correlation coefficient 0.999 (n=6). The lower limit of quantitation (LLQ) was 5 nmol/mL.

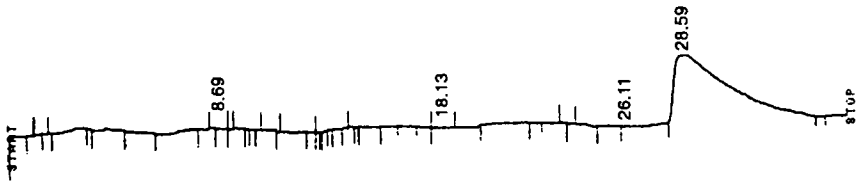

Figure 4: Chromatogram of tranylcypromine authentic sample. Column:Nucleosil C_{18} (250 mm x 4.6 mm I.D., particle size 5 μm); mobile phase; 50 mM KH_2PO_4 (pH 4.55):methanol:water (20:10:70); flow rate: 1 mL/min; chart speed: 0.5 cm/min; temp 23°C; detector UV 264 nm; sensitivity 0.01 aufs; sample quantity: 1 nmol.

Inter and Intra-Assay Accuracy and Precision

The intra-assay accuracy and precision were evaluated by analysing six replicates of two different quality control levels 25 nmol/mL and 250 nmol/mL TCP on the same day. The inter-assay accuracy and precision were determined similarly except that the analysis was carried out several times during one week period. The accuracy of the assay was calculated as the percentage deviation (DEV%) of the mean observed concentration from the nominal concentration of quality control levels. The precision was expressed as the relative standard deviation (RSD%) of the observed concentration from the known concentration of quality control levels. The results were presented in Table 1.

The recovery of TCP is established by analysing a spiked standard solutions of known concentration of TCP onto HPLC system. The recovery is 100%.

To avoid serious cardiovascular crisis due to TCP treatment, a selective, simple and fast method is required to monitor drug level. Accordingly, several analytical methods have been developed to monitor TCP in biological fluid by gas chromatography[7,8] and HPLC with fluorimetric detection.[9] In the investigation reported here, we describe a simple method for TCP determination on Symmetry C_{18} column. The mobile phase used is: 50 mMKH_2PO_4 (pH 4.55): methanol;water (20:10:70). The effluent is monitored at 264 nm. The capacity factor (k`) for TCP is 4.15.

It is of interest to mention that the previous analytical methods performed on plasma samples for pharmacokinetic studies of patient on TCP medication indicated that TCP was rapidly absorbed and eliminated.[7,8,9] The mean time to

Table 1

Accuracy and Precision for TCP Assay

Interassay	No.	Mean	DEV%	RSD%
25	6	24.5	2	6.1
100	6	99.0	1	1.2
Intra-Assay				
25	6	23.5	6	7.23
100	6	98.0	2	2.04

peak plasma level (T_{max}) following a 20 mg oral dose was 1.5 hours and the mean elimination half-life ($t_{½}$) was 2.5 hours.[10] Owing to the depression state of these patient it is advisable to monitor TCP medication in urine. The procedure described herein is applied for the assay of TCP in urine (spiked urine) with a recovery of 75%.

TCP sulfate in pharmaceutical preparation; Parnate tablets, 10 mg (Smith Kline and Beecham, Hertfordshire, England) is determined by the same method. The result of the two batches analysed are 9.0 ± 0.25 and 9.0 ± 0.3 respectively (mean ± S.D).

Repeatability and accuracy are important criteria for the quality of a pharmaceutical analysis, and in HPLC, both are influenced by the quality of the column and the packing material. Materials based on high-purity silica provide superior peak symmetry. The USP tailing factor for 1 nmol of TCP sulfate on Symmetry C_{18} is 1.3 while it is 3.8 for Nucleosil C_{18} column under the same chromatographic conditions (Figure 2 and Figure 4). The short retention time of TCP sulfate assay on Symmetry C_{18} column is also an advantage as it saves time and effort.

CONCLUSION

The use of the Symmetry C_{18} column provides a superior peak shape over a comparable reversed phase C_{18} column for this basic drug (pK_a8.2). Accordingly, more accurate quantitation of the active ingredient in its pharmaceutical formulation can be achieved and validated. The method can also be used in TCP therapeutic drug monitoring in depressive patients. The presented HPLC method is simple, accurate, reproducible and rapid.

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