Quantitative and Qualitative Determination of Temazepam in Pure and Pharmaceutical Samlples by First Order Derivative Spectrophotometry

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Summary:A new and improved first Order Derivative Spectrophotometric method is developed for the simultaneous determination of Temazepam in pure and pharmaceutical samples. Absolute alcohol is used as a solvent through out the study. Temazepam reacts with 0.01% alizirine to give a red-purple colour complex having maximum absorbance at 521.9nm. The colour reaction obeys Beers Law from 4.0-200.0μg/ml of temazepam. The correlation coefficient is 0.99 and the relative standard deviation is 0.84%. The quantitative assessment of tolerable amount of other drugs was also studied. The proposed method can be successfully applied for the quality control of pure temazepam and in pharmaceutical dosage. This method is simple, reliable and reproducible.

Introduction

Temazepam, 7-chloro-1, 3-dihydro-3-hydroxy-1-methyl-5-phenyl-1,4-benzodiazepin-2-one [1], is a Benzodiazepine derivative.(fig.1). It is a hypnotic drug indicated for the relief of INSOMNIA associated with difficulty in falling asleep, frequent nocturnal awakenings, and/or early morning awakenings [2]. There are various analytical studies reported in the literature for the determination of temazepam in pharmaceutical samples and biological fluids by LC/MS [3-4], HPLC [5-7], LC-ESI-MS [8], GC [9], MECC [10]. An alkyl-diol-silica (ADS) extraction column was robust, providing many direct injections of biological fluids for the extraction and subsequent determination of benzodiazepine [11]. A supercritical fluid extraction (SFE) procedure for the analysis of temazepam from whole blood was developed. Quantitative recoveries were obtained by HPLC and results were found to compare well with those obtained by solid-phase extraction techniques [12]. A study is presented for the separation and determination of fifteen 1,4-Benzodiazepine metabolites by capillary electrophoresis (CE) compared with HPLC. The comparison is made by UV detection and LC [13]. However, there is no quantitative analytical method reported in the literature for the determination of temazepam in pure and pharmaceutical samples by using First Order Derivative Spectrophotometry. Therefore, this paper describes a validated method for the qualitative as well as quantitative determination of temazepam.

Results and Discussion

Absorbance Spectrum of Coloured Complex

Temazepam reacts with 0.01% of alizirine solution to give red-purple coloured complex. The absorption First Order Derivative Spectra of which under optimum condition lies at 521.9nm (fig. 3).

Effect of Colour Producing Reagent

It was found that 200μg/ml of Temazepam and 0.8ml of 0.01% Alizirine (0.08mg/10ml) gave the maximum colour (fig. 4). If the concentration of this reagent was changed, the colour intensity diminished. There was no effect of absolute alcohol used for dissolving alizirine and temazepam upon this particular colour reaction.

Effect of Temperature and Heating Time

The colour shows maximum absorbance when heated at 40°C for 120s (fig. 5&6). The colour was also developed at room temperature but was of less intensity. The developed colour remains stable for more than 24 hours.

Effect of Organic Solvents

Different organic solvents such as benzene, hexane, chloroform, methanol and acetone were tested for colour extraction and for stability, but none of them was as effective as absolute alcohol, which was used for dilution of coloured complex up to 10ml.

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Sensitivity

The results for the determination of pure temazepam are shown in the Table 1&2, which shows the sensitivity, validity, and reproducibility of the method. It was also reasonably precise and accurate as the amount taken from identical sample is known and the amount found by the above procedure does not exceed the relative standard deviation 0.84% which is replicate of five determinations. There is no interference of the synthesis of the product of present colour reaction. The optimization has been done at lower analyte concentration. The calibration graph is linear in the range of 0.04-200.0μg/ml. The regression equation was calculated by the method of least squares count from 11 points each of which was the average of five determinations. The correlation between absorbance and concentration is 0.96 in the term of correlation coefficient (r).

Table-1:Determination of Temazepam from Pure Solution.

Temazepam taken (mg/ml)	Temazepam found (mg/ml)	Relative Standard Deviation (%)*
0.1	0.1016	0.84
0.2	0.202	0.50
0.5	0.503	0.31
1.0	1.002	0.58
1.5	1.501	0.47

*Every value is an average of five readings.

Table-2:Comparative Regression Line and Correlation Parameters for the Determination of Temazepam by ($^1D_{521.9nm}$) First Order Derivative Method.

Parameters	Value
Analytical Method	$^1D_{521.9nm}$
Beer's Law Limit (μg/ml)	4.0-200
Molar Absorptivity ($mol^{-1}cm^{-1}$)	1.52×10^3
Regression Equation (Y)*	
Slope (b)	5.0×10^{-3}
Intercept (a)	0.0099
Correlation coefficient (r)	0.99
RSD** (%)	0.84
(Confidence Limit at 95% confidence level)	14.07±0.05

*Y= a + bC Where C is the concentration of analyte (1mg/10ml)
**Calculated from five independent determinations.

Table-3:Quantitative Assessment of Tolerable Amount of Other Drugs.

Drugs	Maximum amount not interfering*(%)
Orphenadrine Citrare	10
Phenol barbitone	30
Brufen	180
Erythrocine	90
Hydroxyzine	70
Metrotieine HCl	20
Atenolol	65

*The value is percentage of drug with respect to 1mg/ml of temazepam, that causes ±0.01 changes in absorbance.

Interferences

The quantitative assessment of tolerable amount of other drugs was also calculated (Table-3) and the recommended procedure for the Derivative Spectrophotometric determination was followed.

Application

The proposed method is successfully applied for the quality control of temazepam in pharmaceutical dosage (Table 4).

Experimental

Apparatus

Cecil CE 7200 UV-Visible Derivative Spectrophotometer with data processing capacity was used. Visible spectra of reference and test solution were recorded in 10mm optical glass cells at a fixed slit width of 2nm.

Midac USA FTIR Spectrophotometer and graduated pipettes were also employed.

Reagents and Solutions

The active substance, Temazepam, was tested for its purity, by checking its melting point (159°C) and FTIR-Spectrum (fig. 7) (which is concordant with the reference structure of Temazepam) and no impurity was found.

Temazepam solutions (1mg/ml) were prepared by dissolving 100mg of temazepam in 100ml of

Table 4:Determination of Temazepam from Pharmaceutical Preparations

Trade name	Pharmaceutical Preparation	manufacturer's specification (mg)	Amount* found (mg)	RSD (%)	Recovery studies (%)
Restoril®	Capsules	15	14.938	0.312	99.6
(Novartis Pharma Pak.Ltd.)	Capsules	30	30.114	0.04	100.38
Hypnotil (PDH Lahore)	Capsule	15	14.992	0.38	99.48

*Every reading is an average of five independent measurements.

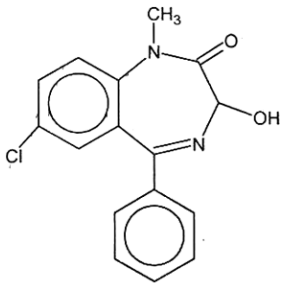

Fig. 1 Structural formula of Temazepam.

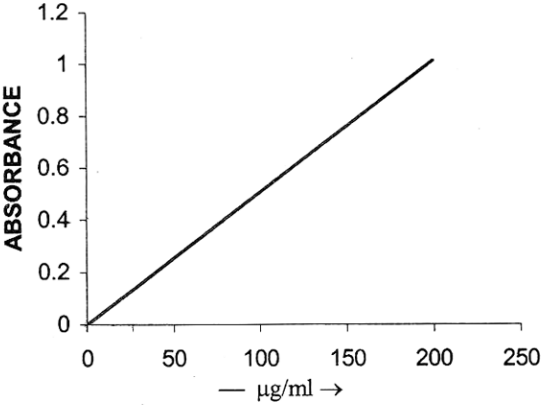

Fig.2 Calibration curve for Temazepam

absolute alcohol to give the stock solutions, which were diluted further as required.

0.01% alizirine solution was prepared by dissolving 0.01gm of it in 100ml of absolute alcohol.

All the chemicals used were of analytical grade.

General Procedure

To an aliquot of temazepam containing 4.0-200µg/ml was added to 0.8ml of 0.01% alizirine solution. The contents were heated for 120s in water bath at 40°C and then cooled. Volume was made up to 10ml with absolute alcohol. The resulting absorbance of red-purple colour was measured at 521.9nm employing alizirine as blank.

The experiment was repeated with different concentration of drug and the calibration curve was prepared (fig.2). The colour reaction obeys Beer's Law from 4.0-200µg/ml of temazepam.

Method for Studying Interferences

To an aliquot (1ml) containing 1mg/ml of temazepam, different amount of various interfering compounds (1mg/ml) (w/v) were added as long as

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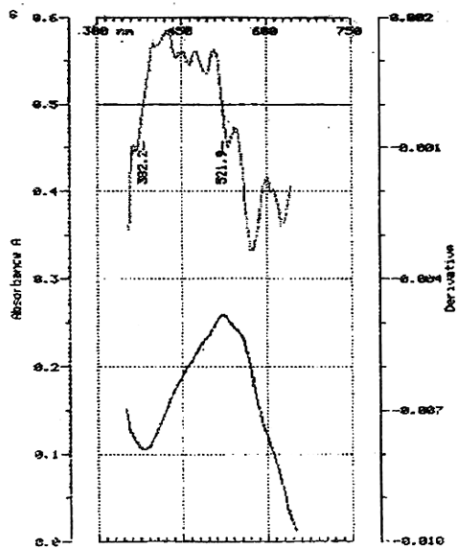

Fig.3 First Order Derivative Spectra of Coloured Complex.

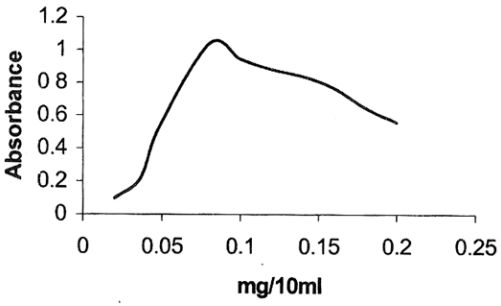

Fig.4 Effect of Alizrine on the stability of Colour.

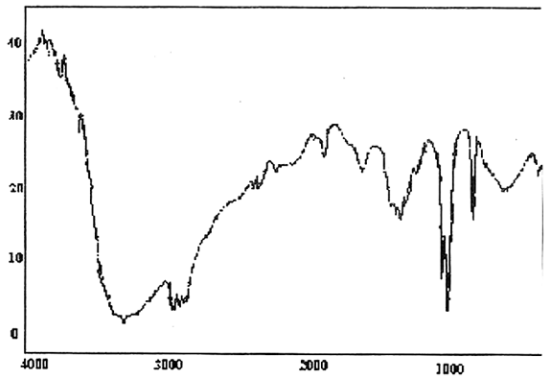

Fig. 7 FTIR Spectrum of Temazepam

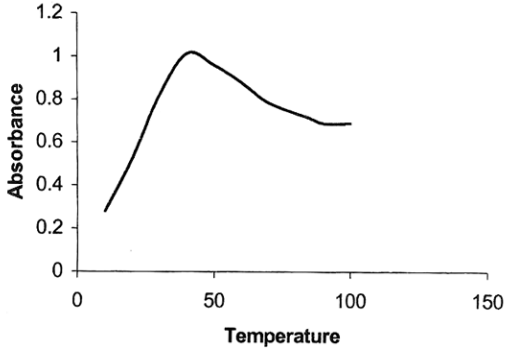

Fig. 5. Effect of Temperature

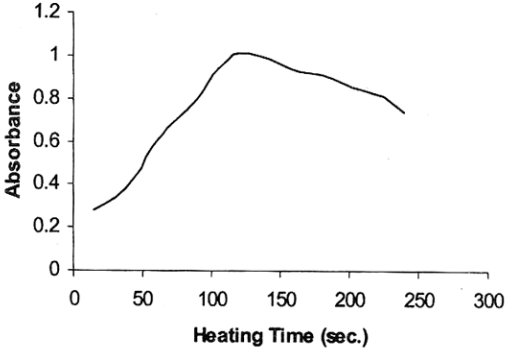

Fig.6. Effect of Heating Time.

solution show ±0.01 absorbance as the pure temazepam solution without addition of interfering organic compound under experimental conditions as described in general procedure. The value was calculated as the percentage of the organic compound with respect to the amount of temazepam.

Method for Determination of Drug in Pharmaceutical Samples

5 capsules of temazepam were dissolved in absolute alcohol. The solution was filtered. Make the volume of filtrate up to 75ml with absolute alcohol to get 1mg/ml of the temazepam solution. An aliquot of temazepam containing 4.0-200µg/ml was taken, the procedure was followed as described above and the absorbance measured at 521.9nm. The quantity per capsule was calculated from the standard calibration curve.

Conclusions

The Derivative Spectrophotometric method for the determination of temazepam is simple, reliable, sensitive, and statistical. It is cheaper and less time consuming as compared to HPLC technique. Like HPLC the present method is precise and accurate also. The method can be successfully applied to the trace determination of drugs in pure as well as pharmaceutical preparation. The literature indicates that this colour reaction has not been reported previously.

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