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Research Article

VALIDATED GC METHOD FOR DETERMINATION OF VALIDOL IN TABLET DOSAGE FORMS

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ABSTRACT

A validated method for the determination of validol in tablet dosage forms using gas chromatography with flame-ionization detection and n-octanol as the internal standard is developed. Validol represents a 25-30% solution of menthol in the menthyl ester of isovaleric acid. The separation of both compounds was achieved on a HP-FFAP (nitroterephthalic acid modified polyethylene glycol) fused silica capillary column, 30 m x 0.53 mm i.d. with 1 µm stationary film thickness. The linearity was established in the range from 0.448 to 2.240 mg/ml for menthol and from 1.152 to 5.762 mg/ml for menthyl isovalerate, respectively. Validation results showed that the method is selective, linear, accurate and precise.

Keywords: validol, gas chromatography, validation, tablet formulation.

INTRODUCTION

Validol tablets are widely used in cardiology as a complex pharmaceutical preparation. The main effect of validol is calming (sedative). Simultaneously, it reflexively slightly dilates blood vessels by stimulation of sensory receptors of the oral mucosa. It removes the headache with stress and after ingestion of nitroglycerin for angina. When validol is taken under the tongue (sublingually), its effect occurs in about 5 minutes [1]. The validol substance contained in the tablets in amounts from 0.054 to 0.060 g is a mixture (1:3) of menthol and its synthetic derivative menthyl ester of isovaleric acid. These substances are highly volatile, therefore gas chromatography (GC) is the preferred method for their quantitative determination. The European Pharmacopoeia recommends GC for determination of related substances in menthol as well as for identification of the substance [2]. Some analytical methods have been reported for assaying validol in pharmaceutical dosage forms. The methods include: gas chromatography [3-5], photometic method [6], polarymetric method [7] and termonephelometric method [8].

In our study, gas chromatographic method with flame-ionization detection was developed and validated [9] for separation, identification and quantitation of menthol and menthyl ester of isovaleric acid in the tablet dosage form. The analytical method described was found to be specific, precise and accurate that could be applied in quality control for the determination of drug in pharmaceutical formulation.

MATERIALS AND METHODS

Chemicals and Reagents

Menthol and menthyl ester of isovaleric acid standards were supplied by Sigma-Aldrich. Tablets Darvalidol were obtained commercially. All other reagents were of analytical grade.

Apparatus and chromatographic conditions

Chromatographic analysis was carried out on a Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) HP 7890 series GC, equipped with flame ionization detector and split/splitless injector. The chromatographic data were recorded by means of HP Chemstation software, which was controlled by Microsoft Windows NT. Separation was performed using a HP-FFAP (nitroterephthalic acid modified polyethylene glycol) fused silica capillary column, 30 m x 0.53 mm i.d. with 1 µm stationary film thickness. In the course of analysis, the column temperature was programmed from 130 to 170ºC at a rate of 30ºC/min. The overall time of analysis was 10 min; the temperatures of the injector and detector were 220ºC and 250ºC, respectively. The flow rate of the carrier gas (nitrogen) was 7.0 ml/min, and the split ratio was 6:2. The volume of a sample was 1.0 µl.

Preparation of solutions

Standard solutions and calibration curves

Standard stock solutions of menthol (5.00 mg/ml), menthyl isovalerate (15.00mg/ml) and n-octanol (10.00 mg/ml, I.S.) were prepared in ethanol. Subsequent dilutions were made in same diluent to achieve the concentrations of calibration solutions in the range 0.448 to 2.240 mg/ml for menthol and from 1.152 to 5.762 mg/ml for menthyl isovalerate. The concentration of the I.S. in the working solution was 1.00 mg/ml.

Sample preparation

Twenty tablets were accurately weighed and finely powdered. The powder equivalent to 300 mg validol was weighed accurately and dissolved in 25 ml ethanol. The solution was filtered through 0.2 µm membrane filter. Five ml of the resulting solution was mixed with 1.00 ml of I.S. (10.00 mg/ml) and was further diluted to 10 ml to get a solution having a concentration of 5.00 mg/ml of validol and 1.00 mg/ml of IS.

RESULTS AND DISCUSSION

Method validation

The proposed method was validated with respect to selectivity, linearity, precision and accuracy to show it could be used for determination of validol in pharmaceutical formulations.

Selectivity

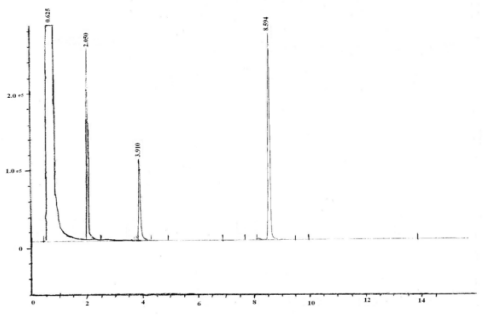

Fig.1:Chromatogram from analysis of sample

From the chromatogram shown in Fig. 1, it is evident, that under the chosen chromatographic conditions, menthol, menthyl isovalerate and n-octanol were completely separated, which indicated that the method is selective and could be used for their simultaneously identification and quantification. Retention times of analytes were shown in Table 1.

The specificity of the GC method was confirmed by injecting blank sample. No other peaks were observed at the retention times of menthol, menthylisovalerate and internal standard, indicating that interfering substances were not present.

Table 1: Retention time of analytes

Peak №	Compound	Retention (min)
1	Ethanol	0.6-0.7
2	N-Octanol	1.8.2.4
3	Menthol	3.7-4.0
4	Menthyl isovalerate	8.4-8.8

Linearity and range

The linearity of the method was determined at six concentration levels ranging from 0.448 to 2.240 mg/ml for menthol and from 1.152 to 5.762 mg/ml for menthyl isovalerate. The standard calibration curves for menthol and menthyl isovalerate were constructed using the analyte/internal standard peak area ratios versus the concentration of the analytes. Linear least-squares regression analysis was performed to assess the linearity as well as to generate the standard calibration equations: $y=Ax+b$, where y is the peak/area-ratio, x the concentration, A the slope and b the intercept of the regression line (Table 2).

Table 2: Linear regression data for calibration curves

Drugs	Menthol	Menthyl isovalerate
Concentration range (mg/ml)	0.448 - 2.240	1.152 – 5.762
Slope	0.65886	0.70487
Intercept	-0.00685	0.00328
Correlation coefficient (r)	0.9978	0.9999

Precision

The precision of the method was evaluated by performing six independent determinations of the test sample preparation and calculating RSD (%). The RSD values measured during assessment of precision were <2.0% for both menthol and menthyl isovalerate, confirming the method is precise (Table 3).

Table 3: Precision of the method

№	Amount found, mg/tablet		
	Menthol	Menthyl isovalerate	Total amount of Validol
1.	16.13	45.41	61.54
2.	16.65	44.25	60.90
3.	16.41	44.70	61.11
4.	16.10	44.12	60.22
5.	15.92	46.32	62.24
6.	16.52	45.26	61.78
Mean	16.29	45.01	61.30
SD	0.281	0.825	0.712
%RSD	1.72	1.83	1.16

Accuracy

Accuracy was studied by adding three different amounts (corresponding to 80, 100, and 120% of the test preparation concentrations) of menthol and menthyl isovalerate to the placebo preparation and comparing the actual and measured concentrations. For each level, three solutions were prepared and each was injected in duplicate.

The results from accuracy study were shown on Table 4.

Table 4: Results from study of accuracy

№	Menthol			Menthyl isovalerate		
	Added amount, mg	Found amount, mg	Recovery, %	Added amount, mg	Found amount, mg	Recovery, %
1.	11.91	11.74	98.57	36.10	36.42	100.9
2.	11.58	12.05	104.1	35.24	35.62	101.1
3.	12.32	12.14	98.54	35.87	35.73	99.61
4.	16.25	16.12	99.20	43.91	43.32	98.66
5.	16.50	16.10	97.58	43.52	43.96	101.0
6.	15.75	15.98	101.5	44.63	44.80	100.4
7.	19.62	19.54	99.59	52.42	52.46	100.1
8.	18.94	19.21	101.4	53.04	52.58	99.13
9.	19.32	19.70	102.0	53.78	53.27	99.05
		Mean:	100.3		Mean:	99.99
		S_d	2.097		S_d	0.922
		RSD (%)	2.091		RSD (%)	0.922

The limits of detection for menthol and menthyl isovalerate were found to be 0.1 mg/ml and 0.5 mg/ml, respectively.

CONCLUSION

The newly developed GC method is specific, precise, accurate and rapid. The analytical procedure is suitable for quality control of pharmaceutical preparation, containing validol.

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