ISSN : 0974-7419 Volume 10 Issue 7

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 10(7) 2011 [453-457]

Development and validation of GC method for the determination of piperazine, 1-methyl piperazine and 1-ethyl piperazine in pharmaceutical drug substances

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Received: 4th November, 2010 ; Accepted: 14th November, 2010

ABSTRACT

A simple GC method was developed for the quantitative determination of piperazine, 1-methyl piperazine and 1-ethyl piperazine in pharmaceutical drug substances like vardinafil and sildenafil. The GC method was developed to separate piperazine, 1-methyl piperazine and 1-ethyl piperazine. The separation was achieved on a DB-17, 30 m, 0.53 mm and 1μm film thickness. The carrier gas used was Helium at a flow of 2 mL/min. The injector temperature and detector temperatures were set at 250°C and 260°C respectively. The injection volume was 1.0 μL. Methanol was used as diluent. The oven temperature was programmed at 150°C for 10 min, followed by 35°C /min up to 260°C for 2 minuets. The developed method was validated with respect to linearity, accuracy, precision, specificity, ruggedness and robustness.

KEYWORDS

Piperazine;
1-methyl piperazine;
1-ethyl piperazine;
Gas chromatography (GC);
Pharmaceutical drug substances;
Validation.

INTRODUCTION

Piperazine, 1-methyl piperazine and 1-ethyl piperazine (Figure 1) were the starting materials in many of the pharmaceutical drug substances like impotence drugs (sildenafil and vardinafil), antipsychotic (olanzapine, Aripiprazole, quetiapine and Ziprasidone), antihistamine drugs (citerazine, cyclizine, cinnarizine and chlorcyclizine), antibiotic drugs (ciprofloxacine), Anthelmintic drugs (diethylcarbamazine) etc. 1-Ethyl piperazine was used as starting material in processes development of vardenafil, presence of trace level of piperazine and 1-methyl piperazine in 1-ethyl piperazine leads to the formation of de ethyl and methyl impurities of vardenafil (Figure 1). De ethyl and methyl impurities of vardenafil were analysed by HPLC[1]. 1-Methyl piperazine was used as starting material in processes development of sildenafil, presence of trace level of piperazine and 1-ethyl piperazine in 1-Methyl piperazine leads to the formation of demethyl (metabolite of sildenafil), dimmer and ethyl impurities of sildenafil (Figure 1). Demethyl, dimmer and ethyl impurities of sildenafil can analyse by HPLC. Formation of these impurities should be controlled by using high pure starting materials (1-Ethyl piperazine and 1-Methyl piperazine). Hence, it was felt necessary to determine piperazine, 1-methyl piperazine and 1-ethyl piperazine in drug substances as well as, piperazine, 1-methyl piperazine in 1-ethyl piperazine and

piperazine, 1-ethyl piperazine in 1-methyl piperazine. Due to the poor chromophore of piperazine, 1-methyl piperazine and 1-ethyl piperazine the detection by UV detector in HPLC was not suitable for the determination of piperazine, 1-methyl piperazine and 1-ethyl piperazine.

A literature survey reveals determination of piperazine by ion exchange chromatohraphy with evaporative light scattering detection[2], gas chromatography/ mass spectrometry, HPLC/ fluorescence[3,4], capillary electrophoresis with indirect UV detection[5], HPLC method with dansyl (DNS) derivatisation[6], using hydrophilic interaction chromatography and evaporative light scattering detection[7] methods were available.

In the present work a simple, cost effective and best GC method was developed by which piperazine, 1-methyl piperazine and 1-ethyl piperazine were well separated with good resolution and peak shapes and the method was validated with respective to precision, specificity, linearity, accuracy and robustness.

EXPERIMENTAL

Materials and reagents

Samples of piperazine, 1-methyl piperazine and 1-ethyl piperazine were purchased from Spectrochem private limited Mumbai, India. Samples of Sildenafil and Vardinafil were received from Process Research Department of Dr. Reddy's Laboratories Limited, Hyderabad, India. HPLC grade methanol, were purchased from Merck, Darmstadt, Germany.

Instrumentation

The GC system, used for method development and method validation was an Agilent 6890 GC system with flame ionization detector. The out put signal was monitored and processed using chemistation software (Agilent).

Chromatographic conditions

The GC column used was DB-17 equivalent to USP Phase G3[(50%-Phenyl)-methylpolysiloxane] (30 m×0.53 mm, 1 μm) capillary column (J & W technologies inc, made in USA). The carrier gas used was helium at a flow of 2 mL/min. The injector temperature and detector temperatures were set at 250°C and 260°C respectively. The detection was by FID detector with air flow of 40 mL/min and Hydrogen flow of 400 mL/min. The oven temperature programmed at 150°C for 10 min, followed by 35°C /min up to 260°C for 2 minuets. Injections were carried out in the split mode with a split ratio of 1:5. The injection volume was 1.0 μL. Methanol was used as a diluent.

Preparation of solutions

(1) Preparation of standard solutions

Standard solution of piperazine, 1-methyl piperazine and 1-ethyl piperazine were prepared by dissolving in diluent and diluting them to the desired concentration. Working solution of 100 μg mL^{-1} of piperazine, 1-methyl piperazine and 1-ethyl piperazine were prepared. Working solutions of 1000 μg mL^{-1} of vardenafil and sildenafil were also prepared in diluent.

Analytical method validation

The developed chromatographic method was validated for selectivity, precision, LOD, LOQ, linearity, range, accuracy, robustness and system suitability[8,9].

Specificity

Specificity of the developed method was assessed by injecting blend solution of piperazine, 1-methyl piperazine and 1-ethyl piperazine along with drug substance.

Precision

The precision of the method was checked by injecting six individual preparations of (100μg mL^{-1}) of piperazine, 1-methyl piperazine and 1-ethyl piperazine blend solution. The %RSD for the area of piperazine, 1-methyl piperazine and 1-ethyl piperazine obtained was calculated.

The intermediate precision (ruggedness) of the method was evaluated by a different analyst and by using different column and a different instrument in the same laboratory.

Limit of detection (LOD) and limit of qualification (LOQ)

The LOD and LOQ for piperazine, 1-methyl piperazine and 1-ethyl piperazine was estimated at a signal-to-noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solutions with known concen-

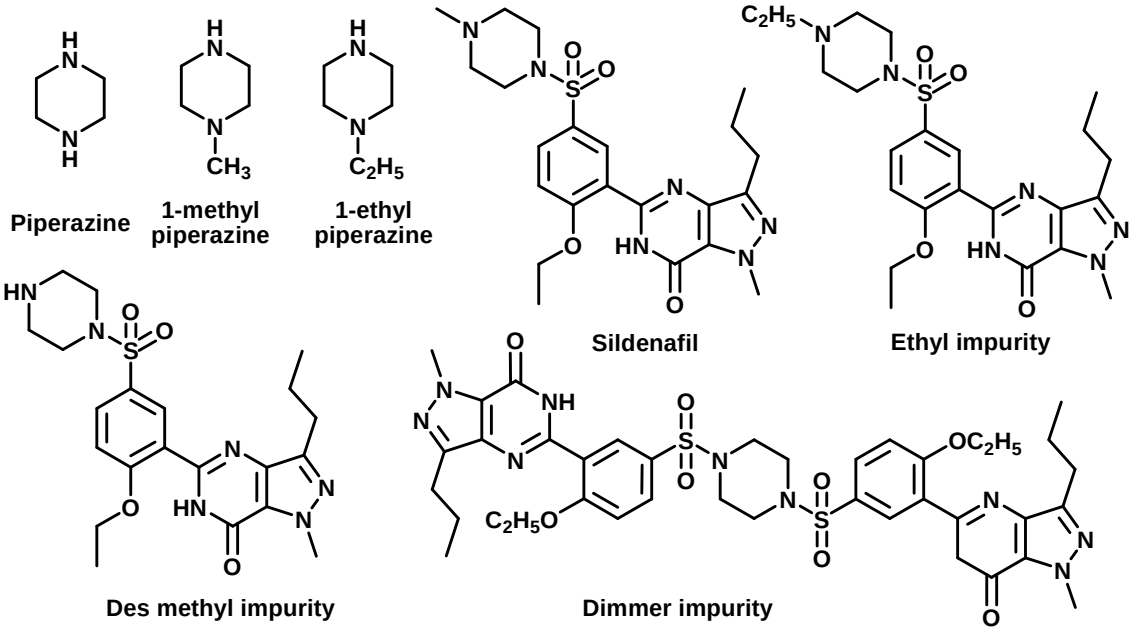

Figure 1 : Chemical structures of piperazine, 1-methyl piperazine, 1-ethyl piperazine, Sildenafil and its impurities

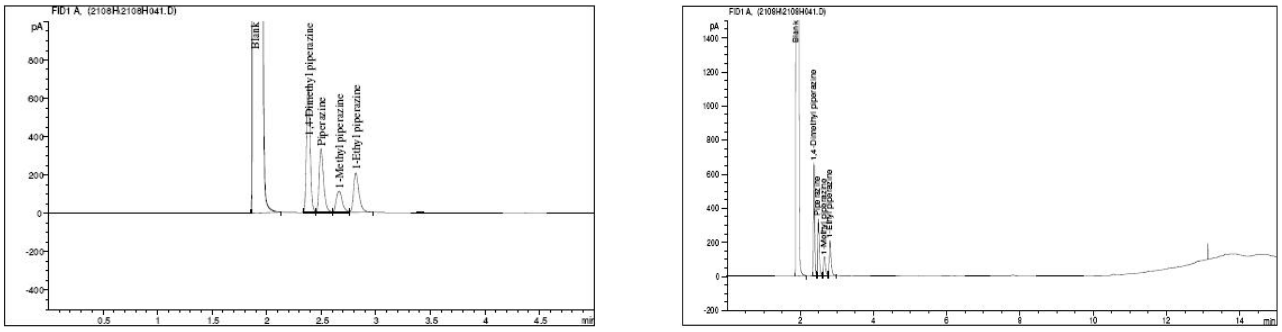

Figure 2 : Typical GC chromatograms of piperazine, N-methyl piperazine, N-ethyl piperazine and N,N-dimethyl piperazine

tration. The precision was checked at LOQ level by injecting six individual preparations of piperazine, 1-methyl piperazine and 1-ethyl piperazine.

Linearity and range

Linearity test solutions for the method were prepared by diluting the piperazine, 1-methyl piperazine and 1-ethyl piperazine stock solution to the required concentrations at seven different concentration levels ranging from LOQ to 200% (permitted maximum level of the impurity) of analyte concentration. The correlation coefficient, slop and Y-intercept of the calibration curve were calculated.

Accuracy

Accuracy study for the method was carried out by spiking piperazine, 1-methyl piperazine and 1-ethyl piperazine in triplicate at 0.075, 0.15 and 0.225% of the analyte (sildenafil) concentration i.e.1000 $\mu g\,mL^{-1}$. The % recoveries for piperazine, 1-methyl piperazine and 1-ethyl piperazine were calculated.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately changed and the resolution between piperazine, 1-methyl piperazine and 1-ethyl piperazine were evaluated. To study the effect of flow rate, it was changed by 0.2 units from 2.0 $mLmin^{-1}$ to 2.2 $mLmin^{-1}$ and 2.4 $mLmin^{-1}$. The effect of injector temperature on resolution was studied by changing it to $\pm$ 5°C from 250°C. The effect of detector temperature on resolution was studied by changing it to $\pm$ 5°C from 260°C. The effect of column oven temperature on resolution was studied by chang-

TABLE 1 : System suitability

Compound	USP resolution	USP tailing factor	No of theoretical plates USP tangent method (N)
1-methyl piperazine	--	1.5	30805
Piperazine	2.0	1.6	28346
1-ethyl piperazine	2.7	1.5	27969

TABLE 2 : Results of intermediate precision

S. No	Parameter	Variation	% RSD	Resolution between 1-methyl piperazine and piperazine	Resolution between piperazine and 1-ethyl piperazine
1	Different system	a) Agient 6890 series	0.8	2.0	2.5
		b) Shimadzu GC-17A series	0.9	2.2	2.7
2	Different column	a) Column-1	0.8	2.0	2.5
		b) Column-2	0.6	2.1	2.8
3	Different analyst	a) Analyst-1	0.8	2.0	2.5
		b) Analyst-2	0.5	2.0	2.8

ing it to ±5°C from 150°C. The effect of injection volume on resolution was studied at 2.0µL instead of 1.0µL.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions

The primary target of the chromatographic method is to get the separation of piperazine, 1-methyl piperazine and 1-ethyl piperazine with good peak shapes. Using different stationary phases like DB-WAX, DB-624 and DB-1, piperazine, 1-methyl piperazine and 1-Ethyl piperazine were analysed separation and peak shapes were not good. DB-17 stationary phase works well for the separation.

The chromatographic separation was achieved on DB-17 equivalent to USP Phase G3 (50%-Phenyl)-methylpolysiloxane) (30 m×0.53 mm, 1 µm) capillary column (J & W technologies inc, made in USA). The carrier gas used was helium at a flow of 2 mL/min. The injector temperature and detector temperatures were set at 250°C and 260°C respectively. The detection was by FID detector with air flow of 40 mL/min and Hydrogen flow of 400 mL/min. The oven temperature programmed at 150°C for 10 min, followed by 35°C / min up to 260°C 2 minuets. Injections were carried out in the split mode with a split ratio of 1:5. The injection volume was 1.0 µL. Methanol was used as a diluent. There was no interference of blank peaks with piperazine, 1-methyl piperazine and 1-ethyl piperazine. In the optimized conditions, piperazine, 1-methyl piperazine and 1-ethyl piperazine were well separated with a resolution of not less than 2.0 and the typical retention times of piperazine, 1-methyl piperazine and 1-ethyl piperazine were about 2.8, 3.0, and 3.2. Analysis was performed for different batches of bulk drug (sildenafil and vardinafil) samples (n = 3).

Method validation

(1) Precision

The % RSD for the area of piperazine, 1-methyl piperazine and 1-ethyl piperazine were within 2.0%. The % RSD for the area of piperazine, 1-methyl piperazine and 1-ethyl piperazine in intermediate precision were with in 2.0% confirming the good precision of the method.

(2) Limit of detection and limit of quantification

The limit of detection of piperazine, 1-methyl piperazine and 1-ethyl piperazine were 0.008, 0.005 and 0.005% (of analyte concentration, i.e., 1000µg mL^{-1}) and the limit of quantification of piperazine, 1-methyl piperazine and 1-ethyl piperazine were 0.03, 0.02 and 0.002% (of analyte concentration, i.e., 1000µg mL^{-1}) for 1 µL injection volume. The precision at LOQ concentration for piperazine, 1-methyl piperazine and 1-ethyl piperazine were below 5%. The recovery at LOQ level found was 98.5, 98.9 and 97.5 respectively for piperazine, 1-methyl piperazine and 1-ethyl piperazine.

(3) Linearity

Linear calibration plot was obtained over the calibration ranges tested, i.e. 25 µg mL^{-1} to 150 µg mL^{-1} and the correlation coefficient obtained was greater than 0.999. The results show that an excellent correlation existed between the peak area and concentration.

(4) Accuracy

The percentage recovery of piperazine, 1-methyl piperazine and 1-ethyl piperazine in bulk drug samples was ranged from 99.0 to 101.0%.

(5) Robustness

In all the deliberate varied chromatographic condi-

tions (flow rate, injector, detector, column temperatures and injection load) the resolution between piperazine, 1-methyl piperazine and 1-ethyl piperazine was not less than 2.0, which confirms the robustness of the developed method.

CONCLUSIONS

The GC method developed for quantitative determination of piperazine, 1-methyl piperazine and 1-ethyl piperazine in bulk drug is precise, accurate and specific. The method was completely validated showing satisfactory data for all the method validation parameters tested. The developed method is simple and can be used for routine analysis.

ACKNOWLEDGEMENTS

The authors wish to thank the management of Dr. Reddy's Laboratories Ltd for supporting this work.

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