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Research Article

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Analytical method for piperazine in an active pharmaceutical ingredient using chemical derivatization and HPLC-UV

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ABSTRACT

A method to form a UV-active derivative of piperazine was developed. The method was based on the reaction of piperazine with NBD-Cl (4-chloro-7-nitrobenzofuran), forming a stable derivative, which was UV-active. The method was used to analyze samples of an active pharmaceutical ingredient (API) for trace amounts of Piperazine. The derivatization approach allowed detection of piperazine at low levels, using readily available HPLC-UV instrumentation. The method was validated with respect to Limit of detection, Limit of quantification, Accuracy, linearity, precision, robust and ruggedness in the range of about 30 to 350 ppm. It was shown through spiked recovery tests that the derivatization reaction occurred smoothly without interference from the API.

Keywords: Piperazine, NBD-Cl (4-chloro-7-nitrobenzofuran), derivatization, Method development

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INTRODUCTION

Diethylenediamine is commonly applied in pharmacy, both as a native compound known under the common name piperazine, and as the starting substance for the synthesis of methyl and hydroxyl derivatives, used for the production of drugs such as estropin, clozapine, or cinarazine. Thus, it should be assayed in many cases, during industrial synthesis as an intermediate product, and as technological impurity of the final products, as well as in the pharmacological and environmental analyses.

Diethylenediamine does not possess chromophores. It absorbs UV light only at a wavelength of 205 nm, and its specific absorption coefficient is very low (0.01). Therefore, the determination of diethylenediamine at a level below 1mg/kg is possible only after its transformation into a derivative with sufficiently high optical density, or emitting induced light. Various primary and secondary aliphatic amines are present in considerable amounts in biological and environmental samples. Their selective removal is theoretically possible, but time- and labor-consuming. The chromatographic properties of coupling products of aliphatic diamines with several carbon

atoms are affected, to a great degree, by the substituent, whose molecular weight is several times higher and which usually possesses unsaturated bonds and an additional heteroatom. Hydrophobic interactions of derivatives of primary and secondary amines do not differ significantly. Additionally, in contrast to secondary amines, derivatives of primary amines may form hydrogen bonds with relevant groups of stationary phases or solid support.

This method has been developed for pharmaceuticals, which do not contain potential co-eluents. Furthermore, the results of chromatographic separation may also be reproducible.

The objective of the present study was the selection of HPLC adsorbents, enabling good separation of NBD Cl (4-chloro-7-nitro benzofurazan) -diethylenediamine and potential co-eluents. The research was carried out for determination of piperazine content in active pharmaceutical ingredients and intermediates.

A variety of techniques have been utilized in detecting and quantitating piperazine, including spectrophotometry,[1] capillary electrophoretic,[2] mass spectrometry [3] and high performance liquid chromatography (HPLC)=fluorescence,[4] and high performance liquid chromatography (HPLC)=UV,[5-9] and HPLC=Evaporative Light Scattering Detection.[10]

EXPERIMENTAL SECTION

Chemicals

Chemicals were reagent grade or better. Acetonitrile (MeCN), methanol (MeOH) and diethyl amine were HPLC grade.Piperazine and NBD-Chloride were purchased from Sigma Aldrich.

Instrumentation

The HPLC-UV system consisted of an Alliance e2695 separations module and 2998 photodiode array UV detector (Waters).

Chromatographic conditions

The Chromatographic separation was achieved on Chiralpak IC (Diacel) of dimensions 250 X4.6 mm, 5µm, maintained at temperature of 35°C. Flow rate was 1.0 ml/min and injection volume was 10µL. Mobile phase consists of mixture of Acetonitrile, Methanol and diethyl amine in the ratio of 90:10:0.1(v/v/v). The mobile phase was filtered through 0.45 µm nylon filter and degassed in ultrasonic bath prior to use. The detection wavelength of 340 nm was used.

Preparation of sample solution

Dissolved 25mg of sample into 50mLvolumetric flask ,dissolve and dilute to volume with diluent (Weigh accurately about 500 mg of NBD Chloride into a 500 ml volumetric flask, dissolve and dilute to volume with Acetonitrile)

Preparation of System suitability solution

Weight accurately about 20 mg of Piperazine standard into a 10 ml volumetric flask dissolve by sonication and make up to the mark with diluent. Transfer 12.5µl of this solution into a 50 ml volumetric flask, dissolve and dilute to volume with diluent.

Method Validation

Precision

The precision of the related piperazine method was checked by injecting six individual preparations of analyte (0.5mg/ mL) spiked with 0.1 % of piperazine with respect to analyte concentration. % RSD of area for piperazine. The intermediate precision of the method was also evaluated using different analyst and different instrument in the same laboratory.

Limit of detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and limit of quantification were determined at a signal to noise of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. Precision study was also carried out at the LOQ level by injecting six individual preparations of piperazine and then calculated the %RSD of the peak area.

Linearity

Linearity test solutions for the related substance method were prepared by dilution of stock solution to the required concentrations. The solutions were prepared at six concentration levels from LOQ% to 150 % of piperazine. Above test were carried out of 2 consecutives days in the same concentration range for related substances method. The % RSD value for the Slope and Y-intercept of the calibration curve was calculated.

Accuracy

The accuracy study of piperazine was carried out in triplicate at 50%, 100% and 150 % of specification level (1000 ppm) to the analyte concentration. The percentages of recoveries for piperazine were calculated from the slope and Y- Intercept of the calibration curve.

Robustness

To determine the robustness of the developed method, experimental conditions were deliberately Altered and the resolution between analyte peak and piperazine peak was recorded. The effect of the methanol ratio in mobile phase preparation studied on resolution by varying by -5 to + 5 %, while other mobile phase components were held constant as stated in Chromatographic conditions. The column temperature was varied by -5 to + 5°C and flow rate of the mobile phase varied from – 0.1 to +0.1 mL/min.

RESULTS AND DISCUSSION

Method Development

The objective of this work was to evaluate the piperazine content for accurate quantification The preliminary trails carried out in reverse phase columns were not fruitful in the separation of the separation of piperazine from API. Different chiral stationary phases were employed during the method development namely Chiralpak IA, Chiralpak IB, Chiralpak ICand Chiral pak AD-H. In Chiralpak IC column was found to be good separation between Piperazine and API peak.Very good resolution was achieved on Chiralpak IC column using mobile phase contains the mixture of Acetonitrile, methanol and DEA (90:10:0.1 v/v/v).The addition of methanol and DEA to the mobile phase plays an important role on enhancing the chromatographic efficiency and resolution between the piperazine and API.

Optimized Chromatographic Conditions

Chromatographic base to base separation was achieved only on a Chiralpak IC (250 x 4.6 mm, 5 microns particle size) chiral column using the mobile phase, which contains the Acetonitrile, methanol and DEA (90:10:0.1 v/v/v).The flow rate of the mobile phase was 1.0 ml/ min. The column temperature was maintained at 35°C and the detection wavelength was 340 nm. The injection volume was 10 µL. The total analysis time for each run was 20 min.

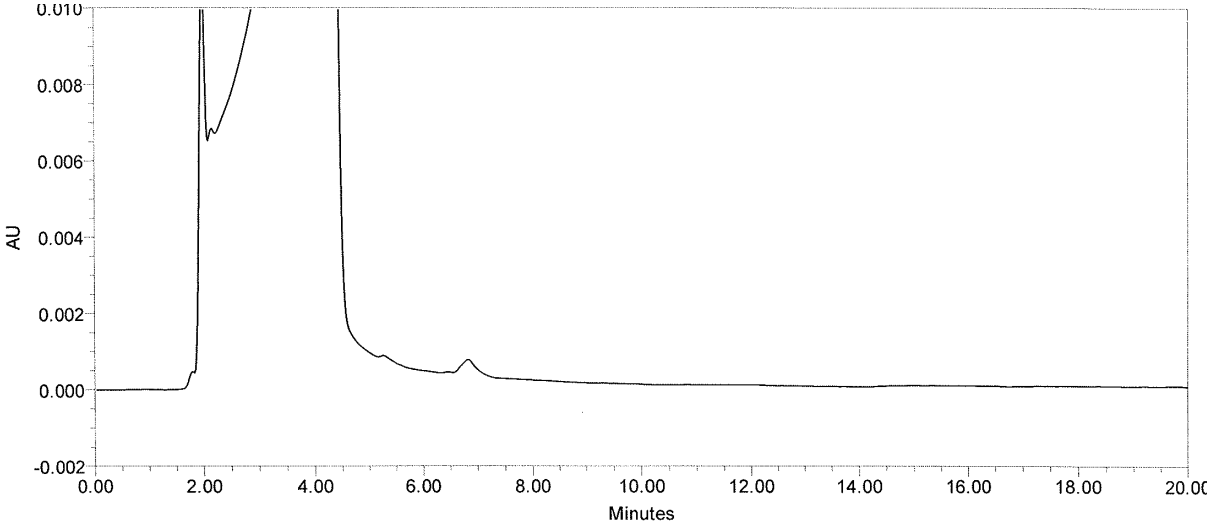

Fig-1: Blank chromatogram

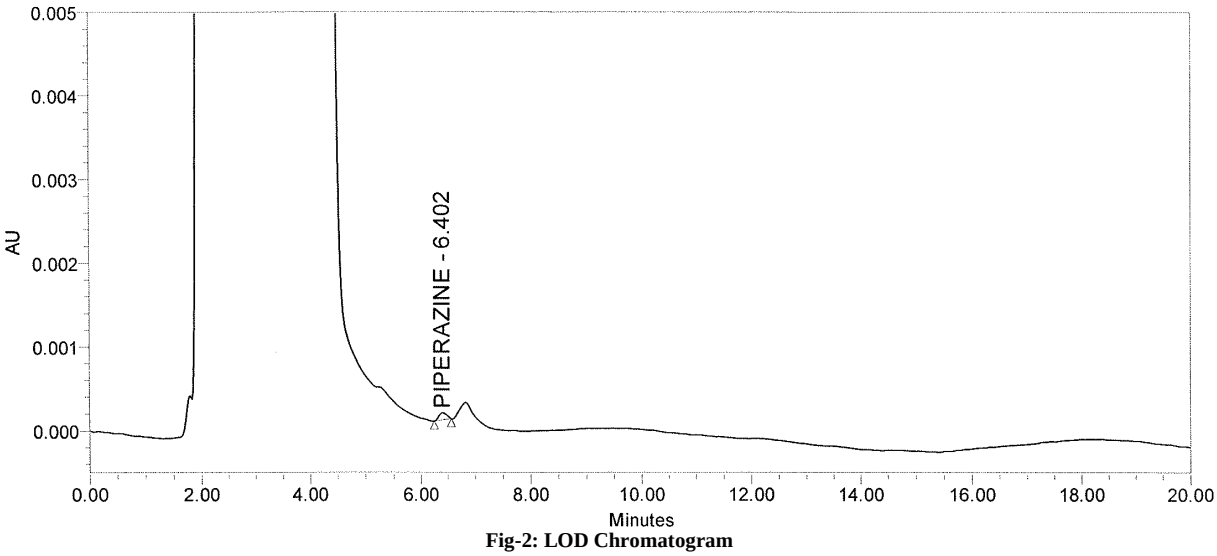

Fig-2: LOD Chromatogram

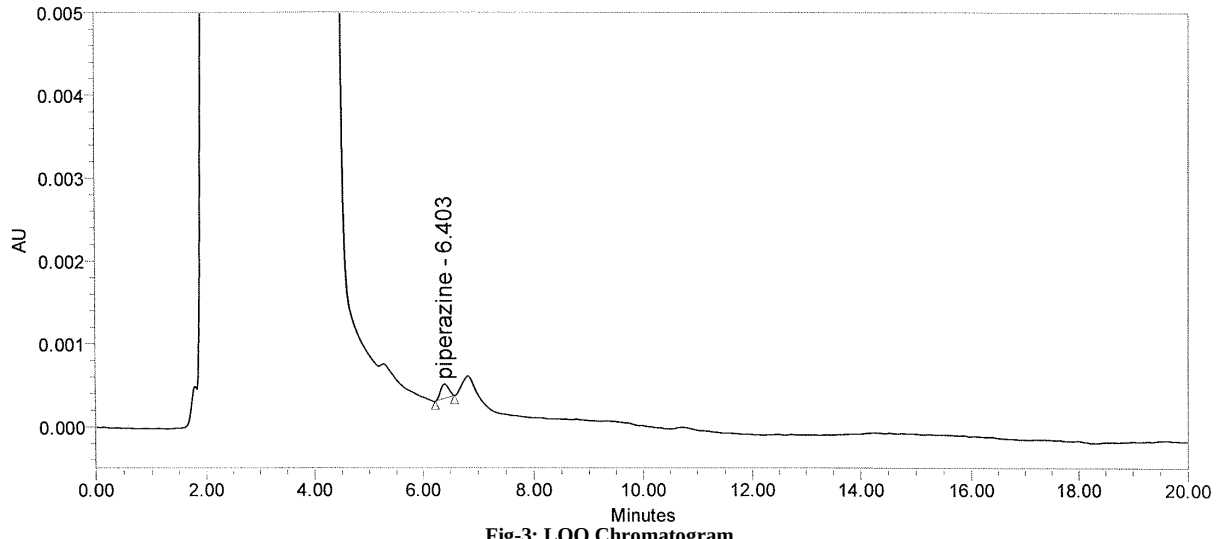

Fig-3: LOQ Chromatogram

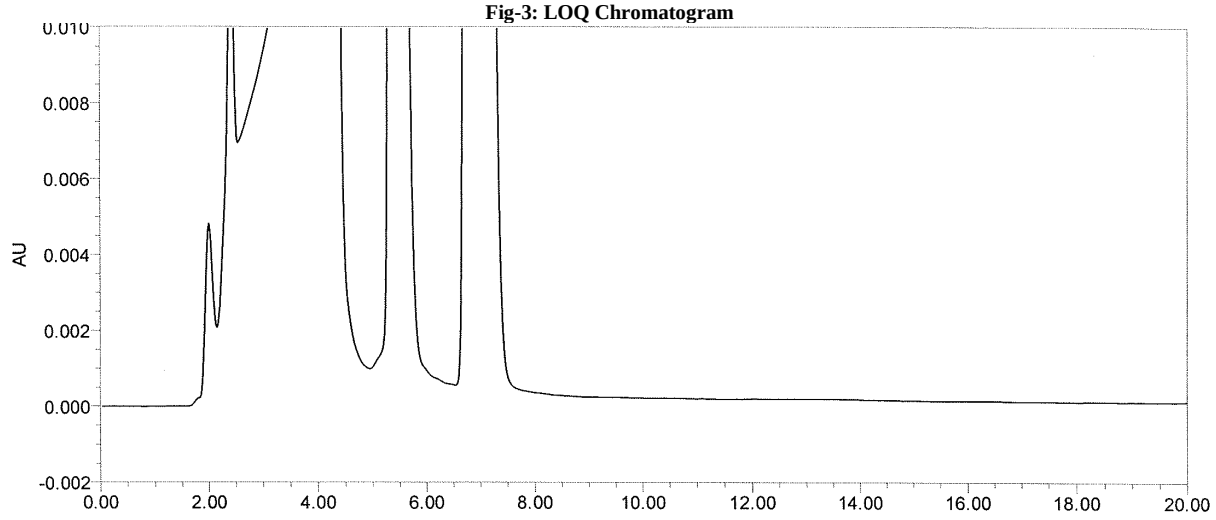

Fig-4: sample chromatogram

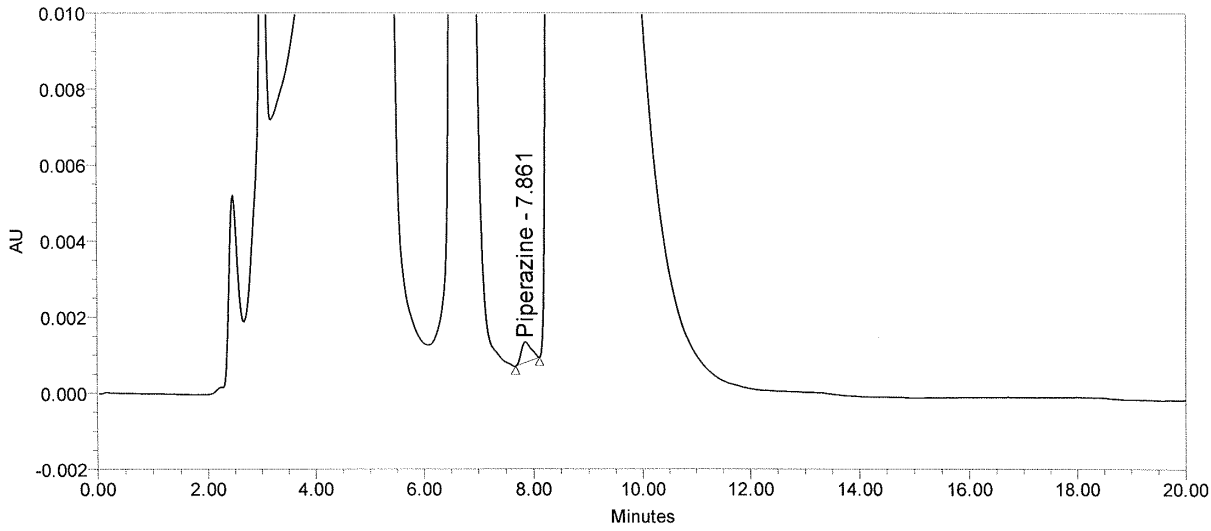

Fig-5: piperazine spiked to sample

Method Validation

Precision

The %RSD for the area of piperazine in method precision study was with in 1.13% conforming good precision of the method.

Limit of detection (LOD) and Limit of Quantification (LOQ)

The limit of detection of piperazine achieved 30 ppm . The limit of quantification of piperazine was 90 ppm.

Linearity

Linear calibration plot for the related substances method was obtained over the calibration range tested i.e. LOQ to 150% for piperazine. The correlation coefficient obtained greater than 0.998. The above results shows that an excellent correlation existed between the peak and the concentrations of piperazine

Table. 1. Method validations Results

Method Validation Data	
Parameter	**Piperazine**
LOD	30 ppm
LOQ	90 ppm
Precision (%RSD)	1.13%
Accuracy (%recovery)	
LOQ	100
50%	108.06%
100%	105.19%
150%	104.87%
Linearity	
corelation	0.999
Slope	61862.398
Intercept	-214.752
% Y-Intercept	-3.49
Robustness	
Different flow	
Idle (1.0ml/min)	1.13%
Low flow (0.8ml/min)	0.7
High flow (1.2ml/min)	1.35%
Different temperature	
Idle (35°C)	1.13%
Low temp. (30°C)	3.56%
High temp. (40°C)	2.52%

Accuracy
The percentage recovery of piperazine in samples varied from 104.87-108.06%.

Robustness
In all the deliberate varied chromatographic conditions (Different flow & column temperature) the %RSD for the area of Piperazine was less than 4.0, illustrating the robustness of the method in table-1.

CONCLUSION

From the above experimental data on the various method validation parameters, it is proved that this method which was designed to determine for Piperazine content by HPLC in PBI is precise, accurate, linear, rugged and robust and range is LOQ to 150% of specification level. Hence, the method can be used for routine application.

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