Determination of 2-(Diethylamino)-*N*-(2,6-Dimethylphenyl) Acetamide in a Gel Pharmaceutical Formulation by High-Performance Liquid Chromatography

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Abstract

An isocratic reversed-phase high performance liquid chromatographic method is developed and validated for the determination of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide (0.3%, w/w) in a gel formulation. The chromatographic separation is achieved with potassium phosphate buffer (pH 7.0)–acetonitrile (47:53, v/v) as mobile phase, a C_{18} column, and UV detection at 254 nm. The calibration curve is linear (r^2 = 1.000) from 20–140% of the analytical concentration of 1.4 µg/mL. The mean percent relative standard deviation values for intra- and interday precision studies are < 1%. The recovery ranges 99.95–100.23% from a gel formulation. The method is specific and successfully routinely used in quality control for the analysis of bulk gel samples and final product release.

Introduction

2-(Diethylamino)-*N*-(2,6-dimethylphenyl) acetamide (Figure 1) is widely used as a local anaesthetic that can be administered in a gel matrix (1). It has also achieved prominence as an antiarrhythmic agent and is now in common use particularly as emergency treatment for ventricular arrhythmias that are encountered after cardiac surgery or acute myocardial infection. Some methods for the determination of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide in gel formulations have been reported, such as spectrophotometry (2–4), gas–liquid chromatography (5), and high-performance liquid chromatography (HPLC) (6–9), but method validation has not been reported. Analytical methods validation is an important regulatory requirement in pharmaceutical analysis. In recent years, the International Conference on Harmonization (ICH) has introduced guidelines for analytical methods validation (10,11) in Japan, Europe, and United States. The most widely applied analytical performance characteristics are accuracy, precision (repeatability and intermediate precision), specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity, range, and stability of analytical solutions. The purpose of this study was to develop and validate a rapid, accurate, sensitive, and simple reversed-phase (RP)-HPLC method for the quantitation of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide in a gel pharmaceutical formulation for bulk and final product release.

Experimental

Chemicals and reagents

All chemicals and reagents were of the highest purity. HPLC-grade acetonitrile, 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide, and potassium phosphate (KH_2PO_4) were obtained from Merck (Darmstadt, Germany). Deionized distilled water was used throughout the experiment.

HPLC instrumentation and conditions

A PerkinElmer (Norwalk, CT) HPLC system equipped with a model series 200 UV–vis detector, series 200 LC pump, series

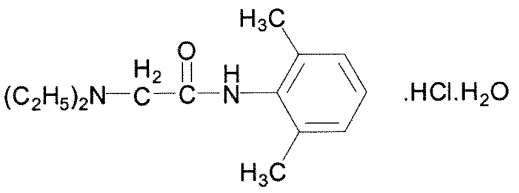

Figure 1. Chemical structure of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide.

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200 autosampler, and series 200 peltier LC column oven were used to chromatograph the solutions. The data were acquired via PE TotalChrom Workstation data acquisition software (v. 6.2.0) using PE Nelson series 600 LINK interfaces. The second instrument used in this study was also a PerkinElmer HPLC system.

The mobile phase consisted of a mixture of a potassium phosphate buffer (pH 7.0)–acetonitrile (47:53, v/v). The flow rate was set to 2.0 mL/min and the oven temperature to 25°C. The injection volume was 20 µL, and the detection wavelength was set at 254 nm. The chromatographic analysis was carried out on a 3.9- × 300-mm i.d., 5-µm C_{18} µ-Bondapak column obtained from Waters (Milford, MA).

Standard preparation

An accurately weighed amount (2.8 mg) of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide reference standard was placed in a 100-mL volumetric flask and dissolved in deionized water (stock). A 5.0-mL aliquot of stock solution was diluted to 100 mL in buffer (pH 7.0), yielding a final concentration of 1.4 µg/mL.

Sample preparation

An accurately weighed amount (400 mg) of sample gel was dissolved in 100 mL buffer (pH 7.0) to provide a concentration of 4000 µg/mL.

Results and Discussion

Method development

The chromatographic analysis of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide (pK_a 7.86) was carried out in the isocratic mode using a mixture of 53% acetonitrile–buffer (pH 7.0; 53:47, v/v) as mobile phase. The column was equilibrated with the mobile phase flowing at 2.0 mL/min for 1 h prior to injection. The column temperature was ambient. Twenty microliters standard and sample solutions were injected automatically into the column. Subsequently, the LC behaviors of both drugs were monitored with a UV detector at 254 nm. Additionally, preliminary precision, linearity, and robustness studies performed during the development of the method showed that the 20-µL injection volume was reproducible, and the peak response was significant at the analytical concentration chosen. Diluting the standard and sample in buffer (pH 7.0) gave solutions that could be injected directly (without further dilution, filtration, or centrifugation). Chromatograms of the 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide gave good peak shape (Figure 2), and coelution of excipients was not observed (Figure 3) at the same retention time as 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide. The retention time for 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide was 2.12 min. System suitability testing was performed by injecting six replicate injections of a solution containing 1.4 µg 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide/mL. The percent relative standard deviation (%RSD) of the peak area responses was measured, giving an average of 0.10 (n = 6). The tailing factor (T) for each 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide peak was 1.05, the theoretical plate number (N) was 8735, and the retention time (t_R) variation %RSD was < 1% for six injections.

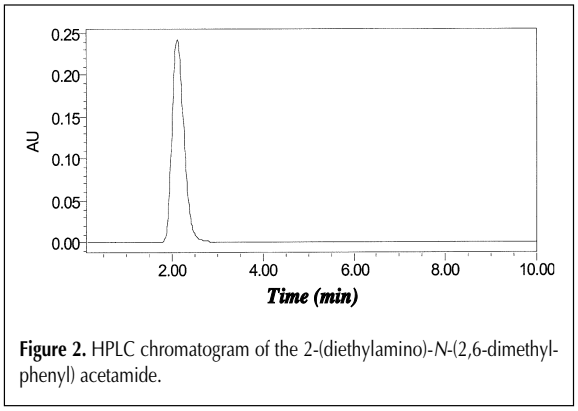

Figure 2. HPLC chromatogram of the 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide.

For the determination of method robustness within a laboratory, a number of chromatographic parameters were determined, which included flow rate, temperature, mobile phase composition, and column from different lots. In all cases, good separations of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide were always achieved, indicating that the method remained selective for 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide component under the tested conditions.

Validation of the method

Stability of analytical solutions

Sample and standard solutions were chromatographed immediately after preparation and then reassayed after storage at room temperature for 48 h. The results given in Table I show that there was no significant change (< 2% response factor) in 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide concentration over this period.

Linearity

Linearity was studied using seven different amounts of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide in the range 20–140% around the theoretical values (1.40 µg/mL), and the following equation was found by plotting peak area (y) versus concentration (x) expressed in µg/mL:

$$y = 1.30664e + 003x \ (r^2 = 1.000) \qquad \text{Eq. 1}$$

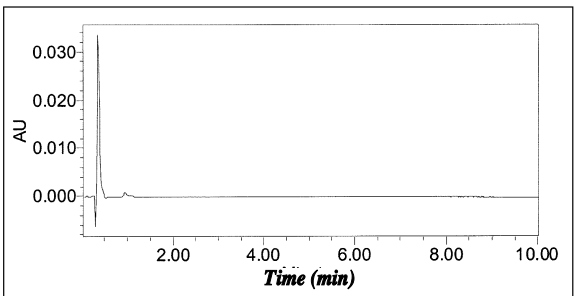

Figure 3. HPLC chromatogram of placebo.

The determination coefficient (r^2) obtained (Table I) for the regression line demonstrates the excellent relationship between peak area and the concentration of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide.

Precision (repeatability and intermediate precision)

The precision of the method was investigated with respect to repeatability and intermediate precision. Repeatability (intraday precision) of the method was evaluated by assaying six replicate injections of the 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide at 100% of test concentration (1.4 µg/mL). The %RSD of the t_R (min) and relative percent peak area were found to be less than 0.19% (Table I).

Intermediate precision (interday precision) was demonstrated by two analysts using two HPLC systems and evaluating the relative peak area percent data across the two HPLC systems at three concentration levels (60%, 100%, and 120%) that cover the assay method range (0.0002–0.014 g/mL). The mean and %RSD across the systems and analysts were calculated from the individual relative percent peak area mean values at the 60%, 100%, and 120% of the test concentration. The %RSD values for both instruments and analysts were < 0.13 (Table I) and illustrated the good precision of the analytical method.

Specificity/selectivity

Injections of the extracted placebo were performed to demonstrate the absence of interference with the elution of the 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide. These results demonstrate (Figure 3) that there was no interference from the other materials in the gel formulation and, therefore, confirm the specificity of the method.

Accuracy/recovery studies

The accuracy of the method was evaluated by adding known quantities of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide in the gel formulation samples to give a range of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide concentration of 75–150% (n = 3) of that in a test preparation. These solutions were analyzed and the amount of analyte recovered calculated. The recovery data expressed as an average percent of triplicate injections are presented in Table II and show good recovery of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide.

LOD and LOQ

The LOD and LOQ tests for the procedure were performed on samples containing very low concentrations of analyte. LOD is defined as the lowest amount of analyte that can be detected above baseline noise, typically, three times the noise level. LOQ is defined as the lowest amount of analyte that can be reproducibly quantitated above the baseline noise, that gives a s/n of 10. The LOD was (s/n 3.2) 100 µg/mL, LOQ was (s/n = 10.2) 250 µg/mL, and %RSD was 0.36% (n = 3).

Table I. Method Validation Results

Validation steps	Parameter	Acceptance criteria	Results
Standard stability	% change in response factors	< 2	0.11
Sample stability	% change in response factors	< 2	0.16
Repeatability (n = 6)	t_R (min) %RSD	2	0.01
	peak area %RSD	2	0.18
Intermediate precision (n = 3)	instruments %RSD	2	0.12
	analysts %RSD	2	0.07
Linearity (n = 7)	correlation coefficient (r^2)	> 0.998	r^2 = 1.000
	intercept	–8.943–6.174	0.142
	slope	0.93–1.081	0.00076
LOD	s/n ratio	s/n = 3:1	(s/n = 3.2), 100 hg/mL
LOQ	s/n ratio	s/n = 10:1	(s/n = 10.2), 250 hg/mL
System suitability (n = 6)	peak area %RSD	< 2	0.10

Table II. Recovery Studies of 2-(Diethylamino)-*N*-(2,6-Dimethylphenyl) Acetamide from Samples with Known Concentration

Sample	Percent of nominal	Amount of analyte (mg) Added	Amount of analyte (mg) Recovered	Recovery (%) (n = 3)	%RSD (n = 3)
1	75	1.70	1.704	100.23	0.13
2	100	3.20	3.204	100.14	0.20
3	150	5.10	5.097	99.95	0.15
Mean				100.10	

Conclusion

An HPLC method for the assay of 2-(diethylamino)-*N*-(2,6-dimethylphenyl) acetamide was developed and validated. The results showed that the method is very selective, no significant interfering peak was detected; accurate, with the percentage recoveries of 99.95–100.23; and reproducible, with the %RSD < 1%. The method was sensitive; as little as 100 µg/mL could be detected with the LOQ of 250 µg/mL. The method was used in quality control for analysis of 2-(diethylamino)-*N*-(2,6-

dimethylphenyl) acetamide in bulk, raw materials, and final gel products pharmaceutical formulations.

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