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Method development and validation for the HPLC assay (potency and related substances) for 20 mg paroxetine tablets

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Abstract

A reversed phase high performance liquid chromatographic (HPLC) method was developed and validated for use as a stability indicating assay (potency and related substances) of paroxetine in paroxetine hydrochloride 20 mg tablets. Assay samples were extracted at a paroxetine concentration of 0.4 mg ml^{-1} utilizing mobile phase as the extraction solvent. The chromatographic conditions employed a C_{18} column (Inertsil, 5 μm, 15 cm × 4.6 mm), isocratic elution with 10 mM 1-decane sulfonic acid sodium salt containing 10 mM sodium phosphate monobasic (pH 3.0)–ACN (60:40, v/v) and ultraviolet (UV) detection at 235 nm.

Keywords: Paroxetine; Liquid chromatography; Assay; Dissolution; Development; Validation; Potency; Related substances

1. Introduction

Paroxetine hydrochloride (paxil, aropax, or seroxat) is an antidepressant agent [1,2] with a structure totally unrelated to other antidepressant agents such as serotonin reuptake inhibitors or tricyclic or tetracyclic agents (Fig. 1). Its action is believed to be linked to the inhibition of neuronal reuptake of serotonin (5-hydroxy-tryptamine, 5-HT) in the central nervous system (CNS). All previous methods for analysis of paroxetine have not been fully validated and involve plasma or serum analyses [3,4] with low sensitivity for possible degradation products (detection at 295 nm). This report describes the development and validation of a stability-indicating method for the assay of paroxetine hydrochloride tablets.

2. Experimental

2.1. Reagents

1-Decane sulfonic acid sodium salt, AR grade, acetonitrile (ACN), HPLC grade, sodium phosphate, monobasic, AR grade, phosphoric acid (85%), AR grade, milli-Q water, and appropriate pH buffers.

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2.2. Equipment

The HPLC system consisted of a Hitachi model L-6200A Intelligent pump, Micromeritics 728 autosampler, Applied Biosystems 785A programmable absorbance detector, ACCESS* CHROM 1.9 chromatography data software with P.E. Nelson A/D Interface, a Valco VICI valve, and a Hewlett Packard 1050 photodiode array detector. A Mettler MT5 micro balance and AE50 analytical balance were used. Gelman Acrodisc 0.45 μm PTFE (25 mm) membrane syringe filters and an Orion model 520A pH meter were also utilized.

2.3. Method development

2.3.1. Selection of mobile phase

The ion pairing mechanism of chromatographic retention on reverse phase columns received a serious consideration during the development process of the chromatographic system of the method because of its potential to achieve chromatography with solid capacity factor and good peak symmetry for paroxetine. In addition, ion-pairing agents were viewed as a selectivity factor that could allow for the retention, elution and resolution of related substances from the active in an isocratic HPLC system.

Perchloric acid (PA), a combination of PA with decane sulfonic acid (DSA) and DSA were evaluated as ion-pairing agents on chromatographic systems that employed an Inertsil C_{18} as the analytical column and ACN as the organic modifier. Chromatography obtained with PA (0.14%, v/v)–ACN (60:40, v/v), however, showed a tailing factor of about 1.2 and a capacity factor of about 2.7 for the paroxetine peak. Chromatograms of paroxetine samples that had been subjected to oxidative conditions and pH extremes, however, showed that the active was not resolved from the degradation products generated by these treatments. Introduction of DSA (10 mM) as a second ion pairing agent increased the retention of the paroxetine peak by about 10 min and showed minimal effect on the retention of the interfering degradation products. The PA was then eliminated from the mobile phase and the stability indicating properties of separations utilizing DSA as the ion-pairing agent were investigated. In an effort to promote the ion-pair formation, the pH of the mobile phase was kept acidic at 3.0 and buffered by phosphate ions (10 mM of monobasic sodium phosphate). Because of the stronger retention of the active in systems with DSA than that shown in systems with PA, the flow rate was increased from 1.0 to 1.2 ml min^{-1}. A mobile phase composition of 10 mM DSA in 10 mM of monobasic sodium phosphate (pH 3.0)–ACN (60:40, v/v) demonstrated chromatography with a solid capacity factor (about 10.0), good peak symmetry (with a tailing factor of about 1.5) and stability-indicating properties for the separation of the degradants generated under forced degradation conditions. It was, therefore, chosen as the elution solvent of the method.

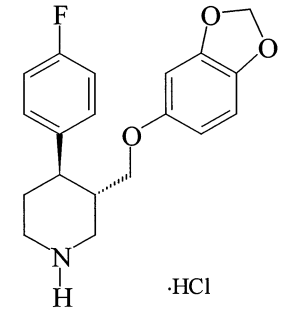

Fig. 1. Structure of paroxetine hydrochloride.

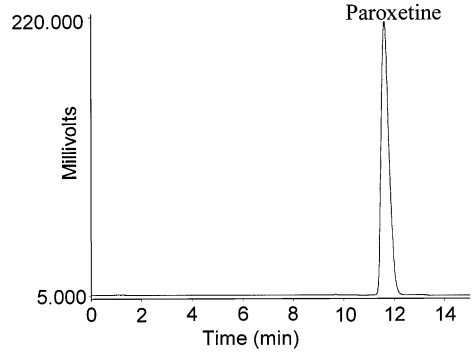

Fig. 2. Example chromatogram of a sample preparation.

Table 1
Range of linearity of paroxetine

% w/w	Concentration (mg ml^{-1})	Measured response	Calculated response	Residual	Response factor
50	0.1999938	2.31347×10^6	2.36634×10^6	-5.287×10^4	1.157×10^7
		2.31665×10^6		-4.969×10^4	1.158×10^7
85	0.3333230	3.94398×10^6	3.90415×10^6	3.983×10^4	1.183×10^7
		3.94224×10^6		3.808×10^4	1.183×10^7
100	0.3999876	4.71441×10^6	4.67306×10^6	4.135×10^4	1.179×10^7
		4.71456×10^6		4.150×10^4	1.179×10^7
135	0.5333168	6.20982×10^6	6.21088×10^6	-1.062×10^3	1.164×10^7
		6.21043×10^6		-4.543×10^2	1.164×10^7
165	0.6666460	7.71801×10^6	7.74870×10^6	-3.068×10^4	1.158×10^7
		7.72270×10^6		-2.600×10^4	1.158×10^7

y-Intercept $= 5.96123 \times 10^4$; slope $= 1.15340 \times 10^7$; correlation coefficient $= 0.999807$; % y-intercept $= 1.3$.

2.3.2. Selection of the extraction conditions

Paroxetine HCl has considerable solubility in water (5.4 mg ml^{-1}) [2] and, therefore, an extraction of tablets with mobile phase was expected to be quantitative and rugged. As the mobile phase makes for an extraction diluent compatible with the chromatographic system, it was chosen as the extraction solvent.

Evaluation of the active peak response of samples extracted at a concentration of 0.4 mg ml^{-1} (25 μl injection volume) indicated that the limit of quantitation of the method would be in the range of 0.1–0.2% of the label claim (LC). In order to avoid challenging the sample capacity of the analytical column, higher extraction concentrations were not evaluated.

The physical handling of the extraction process is based on shaking for a total of 20 min and employs a moderate sonication (10 min) in order to avoid exposing the samples to heat generated from prolonged sonication treatments.

2.3.3. Selection of the detection conditions

Paroxetine exhibited absorbance maxima in the region of 235 and 295 nm with similar intensities. Detection at 235 nm was selected as the detection wavelength in an effort to promote the sensitivity of the method for potentially present unknown degradation products.

2.4. Preparation of mobile phase

The mobile phase was composed of a buffer solution [10 mM 1-decane sulfonic acid sodium salt and 10 mM sodium phosphate monobasic in water (pH 3.0)–acetonitrile (60:40, v/v)]. The buffer solution was prepared by adding 2.44 g of 1-decane sulfonic acid sodium salt and 1.38 g of sodium phosphate, monobasic (monohydrate) to 1 l of water and the pH was adjusted to 3.0 ± 0.1 with 85% phosphoric acid. For mobile phase preparation, 600 ml of the buffer solution and 400 ml of acetonitrile were combined, mixed well, allowed to equilibrate to room temperature, and degassed by helium sparge.

2.4.1. Preparation of standard solution

A working standard solution at a concentration of approximately 0.4 mg ml^{-1} of paroxetine (free base) in mobile phase was prepared in the following manner: due to the lack of paroxetine reference standard, a composite of not less than 20 tablets was prepared by reducing the tablets to a 'fine', uniform particle size powder. After calculating the average tablet weight, composite equivalent to the average weight of two tablets was accurately weighed and transferred into a 100 ml volumetric flask. Approximately 70 ml mobile phase was added and the solution was shaken for 10 min, sonicated for 10 min, and reshaken for 10 min.

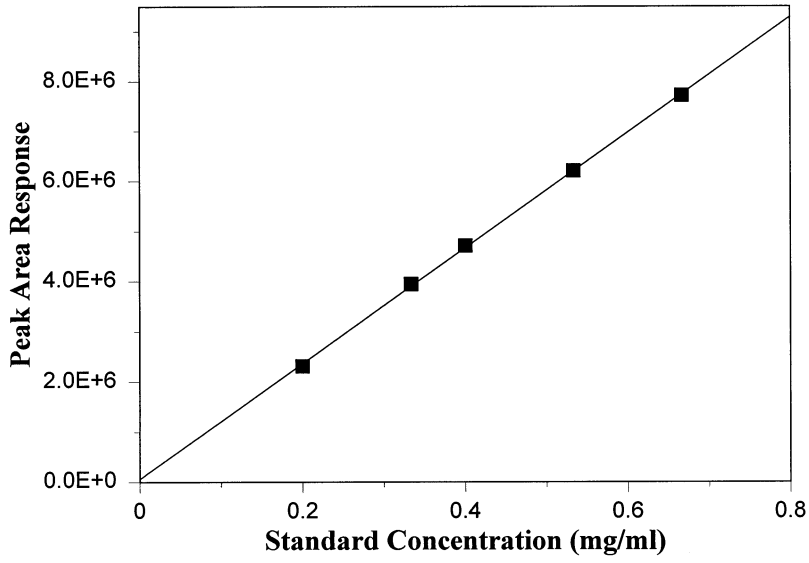

Fig. 3. Range of linearity.

After filling the flask to volume with mobile phase and mixing well, a portion of the solution was filtered through a Gelman Acrodisc PTFE 0.45 μm filter, discarding the first 1–2 ml of the filtrate.

2.4.2. Assay sample preparation

A composite of not less than 20 tablets was prepared by reducing them to a 'fine', uniform particle size powder. After calculating the average tablet weight, composite equivalent to the average weight of two tablets was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 70 ml mobile phase was added, the solution was shaken for 10 min, sonicated for 10 min, and reshaken for 10 min. The flask was filled to volume with mobile phase and mixed well. A portion of the solution was filtered through a Gelman Acrodisc PTFE 0.45 μm filter, discarding the first 1–2 ml of the filtrate.

2.4.3. Chromatographic conditions

An Inertsil C_{18} (15.0 cm × 4.6 mm, 5 μm) column was used at ambient temperature, with UV detection at 235 nm, injection volume of 25 μl and a flow of 1.2 ml min^{-1}. As mentioned previously, the mobile phase consisted of a buffer solution [10 mM 1–decane sulfonic acid sodium salt and 10 mM sodium phosphate monobasic in water (pH 3.0)–acetonitrile, 60:40 (v/v)]. The peak area responses were used for quantitation, and the approximate retention time of paroxetine was 12 min (Fig. 2).

3. Results

3.1. Limit of quantitation (LOQ)

Serial dilutions of a paroxetine sample solution in mobile phase were performed. The dilutions were targeting active concentrations that would result in signal to noise ratios in the range of 8–15:1. A paroxetine concentration of 0.8 μg ml^{-1} (0.2% of the label claim) resulted in an approximate signal to noise ratio of 13:1. The data exhibited a reproducibility of 9.7% (RSD) for triplicate injections at the LOQ level.

3.2. Range of linearity

The linearity of peak area responses versus concentrations were studied from approximately 0.20 to 0.67 mg ml^{-1} for paroxetine. This concentration range corresponds to the approximate levels of 50–165% w/w of the nominal sample active

Table 2
Method repeatability/ruggedness of paroxetine

(a) Sample	Analyst 1		Analyst 2	
	mg Tablet^{-1}	% LC	mg Tablet^{-1}	% LC
1	19.93	99.7	19.89	99.5
2	20.03	100.2	19.82	99.1
3	19.91	99.5	19.91	99.6
4	19.86	99.3	19.93	99.7
5	20.02	100.1	19.92	99.6
6	19.79	99.0	19.90	99.5
Mean (6)	19.92	99.6	19.90	99.5
% RD	0.6	0.6	0.3	0.3
% RSD	0.5	0.5	0.2	0.2
Mean (12)	19.91	99.6		
% RD	0.6	0.6		
% RSD	0.3	0.4		

(b) Summary of chromatographic parameters (Intermediate precision)*

(Analyst)	Tailing factor	Theoretical plates	Capacity factor	Reproducibility (% RSD)	Relative retention** times (RRT)	
					RS1	RS2
1	1.61	4699	9.5	0.1	0.82	0.87
2	1.31	5830	9.9	0.1	0.84	0.89

(c) Method repeatability/ruggedness of related substances[a]

	% LC Analyst 1		Analyst 2	
SAMPLE	RRT = 0.83	RRT = 0.88	RRT = 0.83	RRT = 0.88
1	0.16	<LOQ	0.18	<LOQ
2	0.18	<LOQ	0.18	<LOQ
3	0.15	<LOQ	0.20	<LOQ
4	0.14	<LOQ	0.18	<LOQ
5	0.16	<LOQ	0.18	<LOQ
6	0.17	<LOQ	0.19	<LOQ
Mean (6)	0.16		0.19	
% RD	12.5		5.3	
% RSD	8.8		4.4	
Mean (12)	0.17			
% RD	17.6			
% RSD	9.9			

[a] Relative deviation (RD) between minimum and maximum values.
* Other than RRT, each value is the average of five standard injections.
** Retention time of the related substance (RS) relative to that of paroxetine.

Table 3
Accuracy/recovery for paroxetine HCl

(a) Sample	% Recovery	Mean (3)	%RSD	Level (%)
1	99.5	99.5	0.1	50
2	99.6			
3	99.4			
4	99.7	99.9	0.2	100
5	99.9			
6	100.0			
7	99.4	99.6	0.2	150
8	99.5			
9	99.8			
Mean (9)	99.6			
% RSD	0.2			

(b) Accuracy/recovery for paroxetine HCL related substances

Sample	% Recovery Imp # 1 (RRT = 0.83)	Mean (3)	%RSD	Level
1	0.15	0.15	3.9	50
2	0.14			
3	0.15			
4	0.14	0.14	7.1	100
5	0.15			
6	0.13			
7	0.14	0.14	0.0	150
8	0.14			
9	0.14			
Mean (9)	0.14			
% RSD	4.7			

concentration. The data (Table 1, Fig. 3) meet the acceptance criteria for a correlation coefficient ≥ 0.999 and a y-intercept of $\pm 2.0\%$.

Linearity at the 0.2% level (0.8 μg ml^{-1}) was established by assaying replicate injections of a paroxetine HCl solution at that level. The mean assay value was found to be 0.19% (95% of the theoretical value) with a %RD of 0.2%.

3.3. System repeatability

The system repeatability was assessed from ten replicate injections of a sample solution of paroxetine at the analytical concentration of about 0.4 mg ml^{-1}. The RSD for the active was found to be 0.1%, and for the two related substances 4.1% and $<$ LOQ, respectively.

3.4. Method repeatability/intermediate precision

Method repeatability/intermediate precision was assessed from the assay of six samples by two different analysts using different chromatographic systems on different days. The chromatographic parameters and the results for the active and the related substances are summarized in Table 2a–c. The assay method repeatability/intermediate precision acceptance criteria set in the validation were that for each data set (analyst) and for all the data combined the RSD of the potency (mg per tablet) $\leq 2.0\%$; relative deviation (RD) of minimum and maximum observation $\leq 2.0\%$. The related substances method repeatability/intermediate precision acceptance criteria set in the validation were: (a) if mean total related substances is $\geq 1.0\%$, for each set (analyst) of data

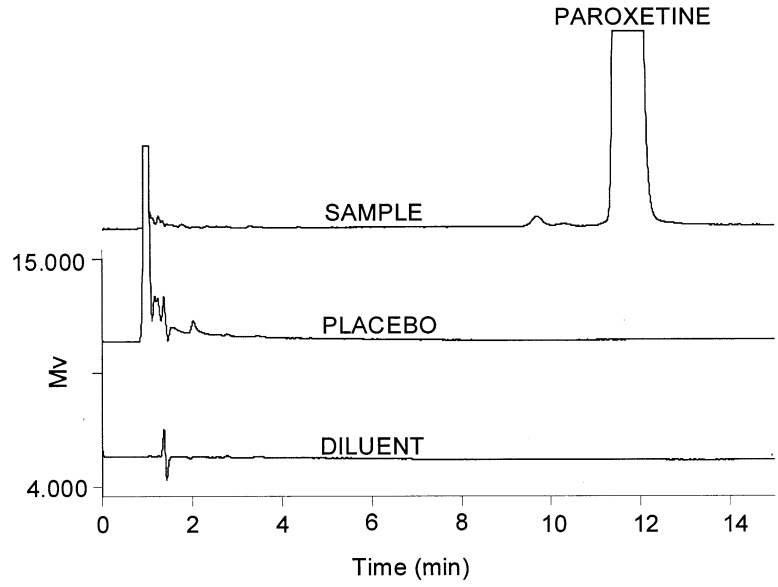

Fig. 4. Overlay of chromatograms of paroxetine sample, diluent, and a placebo solution.

and for all data combined% RSD $\leq 15.0\%$; RD of minimum and maximum observation $\leq 15.0\%$; (b) if mean total related substances is between 0.5 and 1.0%, for each set (analyst) of data and for all data combined% RSD $\leq 20.0\%$; RD of minimum and maximum observation $\leq 20.0\%$; and (c) if mean total related substances is less than 0.5%, the results for both analysts are generally considered equivalent. The data of Table 2a–c meet these acceptance criteria.

3.5. Accuracy/recovery

The excipients in the tablets used in this validation study contained the following inactive ingredients: dibasic calcium phosphate dihydrate, hydroxypropyl methylcellulose, magnesium stearate, polyethylene glycols, polysorbate 80 (Tween 80), sodium starch glycolate, and titanium dioxide. The recovery of paroxetine hydrochloride from the tablets was studied at three different levels, corresponding to 50, 100, and 150% of the nominal analytical concentration. The mean recovery data obtained for each level as well as for all levels combined (Table 3a–b) were within 2.0% of the label claim for the active, which satisfied the acceptance criteria set for the study. The first

Table 4
Extraction robustness study

Amount of ACN in extraction solvent:	42.5%	37.5%
Preparation	% LC	% LC
1	99.2	99.9
2	100.1	99.2
3	100.0	99.6
Mean (3)	99.8	99.6
% RSD	0.5	0.4

Table 5
Comparisons of filtered and centrifuged samples[a]

Preparation	% Paroxetine recovery	% Recovery RS 1 (RRT 0.82)
1	99.9	102.1
2	100.2	99.6
3	100.1	97.4
Mean (3)	100.1	99.7
% RSD	0.2	2.4

[a] Related substance (RS) 2 (RRT = 0.88) was detected at less than the limit of quantitation in both centrifuged and filtered samples.

Table 6
Stability of paroxetine and related substances in analytical solutions[a]

Time (days)	% of the Initial	RRT 1	Related substances		
			% LC	RRT 2	% LC
Initial		0.82	0.16	0.87	<LOQ
1	99.7	0.84	0.17	0.89	<LOQ
2	100.3	0.83	0.18	0.88	<LOQ
3	100.0	0.84	0.14	0.89	<LOQ
6	100.2	0.83	0.15	0.88	<LOQ

[a] Acceptance criteria: 'Assay': potency of aged preparation = fresh ± 2.0%. Related substances: A. If total% LC of fresh is ≥1.0%, LC of aged preparation = fresh ± 15.0%; B. If total % LC of fresh is between 0.5 and 1.0%, LC of aged preparation = fresh ± 20.0%; C. If total% LC of fresh is ≤0.5% and it does not increase over 0.5% in the aged solution, then the aged solution is generally considered stable.

unknown impurity with relative retention time (RRT) of 0.83 was quantitatively recovered from all three levels, whereas the second impurity (RRT = 0.88) was detected in all sample preparations at levels below the limit of quantitation.

3.6. Specificity

Injections of diluent and placebo tablets showed no interferences with the elution of paroxetine (Fig. 4).

3.7. Extraction robustness study

Triplicate sample preparations employing increased level of organic and extended shaking and sonication time relative to the nominal extraction conditions of the method were performed. Triplicate sample preparations employing decreased level of organic and reduced shaking and sonication time relative to the nominal extraction conditions were also carried out. The mean potency data obtained with each of the modified extraction treatments (Table 4) were within 1.5% of the

Table 7
Degradation of paroxetine 20 mg tablets

Condition	Time (h)	% Recovered	RRT* of degradation products
Acid 0.1 N HCl, 80°C	50	98.0	None detected
Base 0.1 N NaOH, 80°C	50	96.5	1.65
Hydrogen peroxide 0.3%, 80°C	2	73.0	0.19, 0.22, 0.23, 0.27 0.31, 0.35, 0.39, 0.43 0.45, 0.49, 0.52, 0.55 0.59, 0.63, 0.71, 0.76 1.13, 1.36, 1.64
Heat dry, 80°C	72	99.2	None detected
Heat wet, 80°C	72	96.2	1.65
Light dry, 1000 foot candles	144	99.5	None detected
Light wet, 1000 foot candles	144	80.3	0.17, 0.20, 0.23, 0.42 0.44, 0.55, 0.82, 0.88 1.13, 1.60

* RRT, relative retention time.

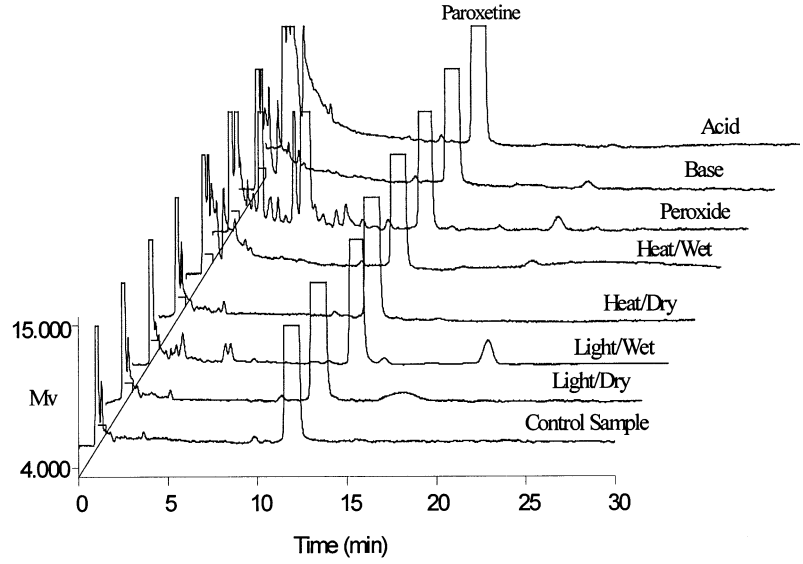

Fig. 5. Overlay of degradation study chromatograms.

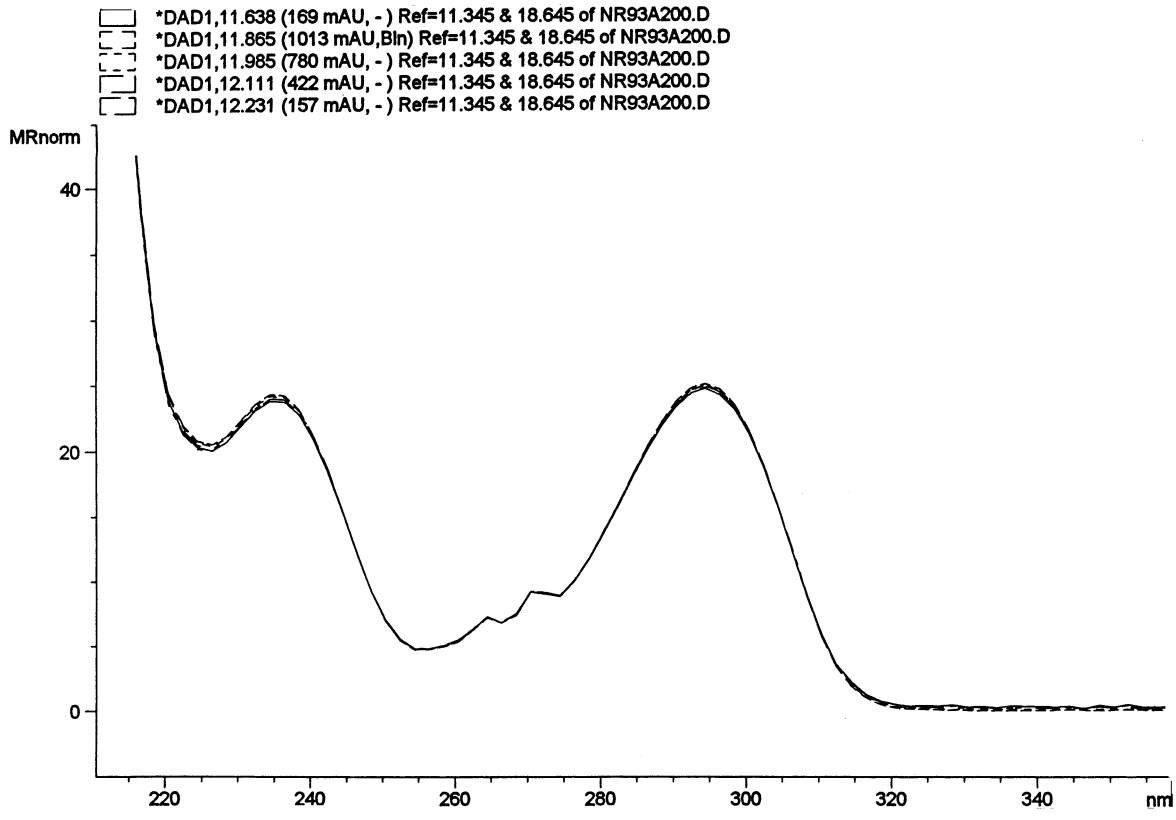

Fig. 6. Example diode-array spectrum of a sample preparation.

mean potency data obtained for the method repeatability/intermediate precision studies (Table 2) which satisfied the acceptance criteria for extraction robustness.

3.8. Filter study

The filtration process of the method was qualified by comparing three separately filtered portions of a sample preparation against a portion of the same solution which was clarified by centrifugation for 15 min at 3000 rotation per min. The acceptance criteria for the filtration study were: filtered solution = centrifuged $\pm$ 1.5%. The data of Table 5 met the acceptance criteria for the filtration study.

3.9. Stability of analytical solutions

Stability of paroxetine and its related substances in analytical solutions was evaluated by assaying a sample solution immediately after its preparation and then, against freshly prepared standards, as it aged for 6 days at room temperature while protected from light (Table 6). The data of Table 5 met the acceptance criteria for a 6-day period.

3.10. Degradation studies

Forced degradation studies were performed to provide an indication of the stability-indicating properties and specificity of the procedure. The degradation samples were prepared by transferring approximately 300 mg (equivalent to one average tablet weight) of sample composite into 50 ml volumetric flasks. Intentional degradation was attempted using acid, base, hydrogen peroxide, heat, and light. After the degradation treatments were completed, the samples were allowed to cool to room temperature and prepared according to assay sample preparation, after being neutralized with acid–base (if needed). The samples were analyzed against a control sample (no degradation treatment). The percentage of paroxetine recovered is shown in Table 7. Degradationpeaks, where observed, were resolved from the paroxetine peak. Spectra taken during the upslope, apex, and downslope did not reveal any degradation products or impurities coeluting with the paroxetine peak. An overlay of the degradation study chromatography and a representative diode-array spectrum of a sample preparation are shown in Figs. 5 and 6, respectively.

4. Conclusions

The linearity of the paroxetine peak area responses was demonstrated from approximately 50 to 165% of the working analytical concentration of 0.4 mg ml^{-1} by a correlation coefficient of 0.9998 and a y-intercept of 1.3%. The paroxetine limit of quantitation (LOQ) was 0.8 μg ml^{-1} [label claim (LC) = 0.2%]. The precision of the paroxetine chromatographic response was calculated from ten replicate injections of a sample solution prepared at the nominal analytical concentration and showed a relative standard deviation (RSD) of 0.1%. Variations in the level of organic and the length of the physical treatment employed in the sample extraction had no effect on the extraction of paroxetine. Method repeatability for each strength was assessed from six sample preparations by two different chemists on 2 different days utilizing different chromatographic systems. The mean mg per tablet was 19.91 (RSD 0.3%), for the 12 preparations.

According to a recovery study performed 50, 100, and 150% of the analytical concentration, the extraction of the active, as well as its related substances, was shown to be quantitative.

Forced degradation studies showed that paroxetine elutes as a spectrally pure peak resolved from the degradation products of the formulation, thus demonstrating the stability indicating properties of the method. It should be noted that a variation of the assay method has been developed and validated for the dissolution of paroxetine hydrochloride tablets in simulated gastric fluid without pepsin.

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