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Stability Indicating Spectrophotometric Methods for the Determination of Oxetacaine in the Presence of Its Hydrolytic and Oxidative Degradation Products

Eman S Elzanfaly¹, Enas A Amer², Sara A Galal², Hala E Zaazaa¹

¹Analytical Chemistry Department, Cairo University, Cairo 11562, Egypt

²National Organization for Drug Control and Research (NODCAR), Giza 35521, Egypt

ABSTRACT

In this study, the overlapped spectra of Oxetacaine (OXT), its hydrolytic and oxidative degradation products were well resolved using two simple spectrophotometric methods. The first method depends on measuring the peak amplitudes of first derivative of the ratio spectra (1DD) at 224.3 for selective determination of Oxetacaine in the presence of its degradation products. While in the second method, it was determined by measuring the difference between amplitudes at 216.1 nm and 227.9 nm of the recorded ratio spectra. All the developed methods were validated according to ICH Guidelines, OXT was determined with acceptable accuracy and precision.

Keywords: Oxetacaine, Mephentermine, Ratio difference

INTRODUCTION

Oxetacaine (OXT) (Figure 1) is 2,2'-(2-hydroxyethylimino) bis [N-(α,α -dimethylphenethyl)-N-methylacetamide]. It is a local anaesthetic used for the relief of pain from gastritis, duodenal ulcers, hiatus hernia and peptic esophagitis [1]. OXT is an official drug in British pharmacopoeia which analyse it by a potentiometric method [2]. The literature survey reveals that OXT was determined by GC-MS method to confirm Urinary Excretion of OXT metabolites Mephentermine and Phentermine, two substances prohibited in sports by the World Anti-Doping Agency, Following the Ingestion of OXT [3]. Also Rendic [4] proposed another GC-MS method to structurally determine both the parent drug and its metabolites in urine and the effect of inhibition and induction of drug metabolism by other drugs administered concomitantly. Unterhalt and Wenning [5] proposed HPLC method to separate OXT metabolites. Also Hsu et al. [6] applied LC-MS-MS to analyze the excretions of three volunteers who ingested OXT and presented positive results for mephentermine and/or phentermine to prove OXT as a source of mephentermine and phentermine in athletes' urine specimens.

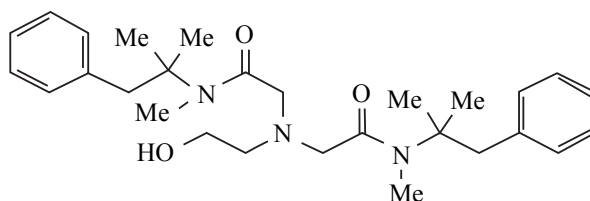


Figure 1: Chemical structure of oxetacaine

OXT and its metabolites were also monitored by Lee *et al.* [7] in rat plasma using liquid chromatography–tandem mass spectrometry after OXT administration, and compared it with phentermine itself after phentermine administration to clarify the relationship between phentermine production by OXT ingestion and the possible OXT dependence. They also determined OXT metabolites by LC–MS/MS in rat hair after OXT administration to investigate the possibility of phentermine detection in hair from OXT abusers.

The International Conference on Harmonization (ICH) guideline entitled ‘stability testing of new drug substances and products’ requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance [8].

No method has been reported in the literature for the determination of OXT in presence of its degradation products. Therefore, it was thought necessary to study the stability of OXT towards acidic, basic and oxidative processes so the aim of this work was to develop stability indicating methods for determination of OXT in presence of its degradation products using the first derivative of the ratio spectra (¹DD) and ratio difference spectrophotometric methods.

EXPERIMENTAL

Instrumentation

SHIMADZU dual beam (Kyoto, Japan) UV-visible Spectrophotometer model UV-1650 PC connected to IBM compatible and an hp 1020 laser jet printer. UV probe 2.21 software was used. The spectral band width 2 nm was and scanning speed was 2800 nm/min. The absorption spectra of a test and reference solutions were recorded in 1 cm quartz cells over the range 200–400 nm and Water-bath of lab house (model SHWB -30).

Materials and reagents

Oxetacaine working standard was obtained as kind gift sample from Eipico Company (Cairo, Egypt). Its purity was labelled to be 99.9% and confirmed by analysis according to official method [2].

Concentrated hydrochloric acid; Honeywell Burdick & Jackson, USA (1 N HCl was prepared in methanol), Sodium hydroxide; El Nasr company for chemicals (1 N NaOH was prepared in 3 mL water to solubilise NaOH and then complete to volume with methanol), Hydrogen peroxide 30% (Panreac, Spain).

Mucogel[®] suspension (Mucogel[®], Batch No. 1200472, label claim: 0.2 g/100 mL) manufactured by EIPICO, Egypt were purchased from local market.

Standard solutions

An accurately weighed 5 mg of Oxetacaine was transferred into 100 mL volumetric flask and dissolved in methanol (final stock concentration 50 µg/mL).

Preparation of the degradation products

All degradation stock solutions were derived from complete degradation of 1 mg/mL of OXT standard solution.

Preparation of hydrolytic (acidic or alkaline) degradation products

Fifty milligrams of Oxetacaine standard was dissolved in 1 N methanolic HCl or 1 N methanolic NaOH, refluxed in 250-mL round-bottom flask for 10 and 6 h, respectively. The degraded solution was then cooled, neutralized with an amount of acid or base equivalent to that of the previously added till pH was 7. The solution was nearly evaporated to dryness, cooled, transferred into a 50-mL volumetric flask with methanol. Then the volume was completed to the mark with the same solvent and filtered to obtain solution with final concentration of 1 mg/mL.

Preparation of oxidative degradation product

Twenty five milligrams of Oxetacaine was dissolved in methanol, refluxed in 250-mL round-bottom flask with 5 mL 30% H₂O₂ for 9 h, evaporated by rotary evaporator then the residue was dissolved in methanol to obtain a final concentration of 1 mg/mL.

PROCEDURES

Spectral characteristics

The absorption spectra of standard solution OXT and its degradation products were recorded over the range 200–400 nm using methanol as blank.

Experimental conditions and construction of calibration curves

For (¹DD) method, aliquots of OXT stock solution (50 µg/ mL) equivalent to 40–200 µg/mL were accurately transferred into a series of 5-mL volumetric flasks then diluted to volume using methanol. ¹DD curves were recorded at $\Delta\lambda=8$ nm and scaling factor=10. The absorption spectra of this solution were divided by the absorption spectrum of 40 µg/mL of the hydrolytic degradation products (as a divisor), and then the obtained ratio spectra were differentiated with respect to wavelength. The peak amplitudes at 224.3 nm were recorded. Calibration graph was constructed relating the peak amplitudes of (¹DD) to the corresponding concentrations. The regression equation was then computed for the studied drug at the specified wavelength and used for determination of unknown samples containing OXT.

For ratio difference method, aliquots equivalent to 10-200 μg were separately transferred from OXT standard stock solution into a series of 5-mL volumetric flasks, diluted to volume with methanol to give a concentration range of 2-40 $\mu\text{g}/\text{mL}$. The zero order spectrum ($^{\circ}\text{D}$) of each dilution was recorded against blank then divided by the stored zero order absorbance spectrum of 40 $\mu\text{g}/\text{mL}$ of the hydrolytic degradation products' stock solution. The difference between amplitudes at 216.1 nm and 227.9 nm of the recorded ratio spectra was measured and used for construction of calibration curve by plotting the ratio difference against concentration of OXT being examined, and the regression equation was computed.

Analysis of OXT in Mucogel[®] suspension

Mucogel[®] suspension bottle was shaken well then 2.5 mL was accurately transferred into a 100 mL conical flask, 50 mL of dichloromethane was added to extract OXT only and exclude any interference of the excipients in the recorded spectra, sonicated for 30 minutes and then filtered through 0.45 μm membrane filter. The filtrate was then evaporated till dryness in warm water bath. The obtained residue was reconstituted with methanol in 100 mL volumetric flask and the volume was completed with methanol. Aliquots equivalent to 40, 120 and 200 μg were accurately transferred into a series of 5-mL volumetric flasks. The volume was completed with methanol. The procedures previously described under experimental conditions were followed. The concentrations of OXT were calculated from the corresponding regression equations.

RESULTS AND DISCUSSION

Method development and optimization

None of the reported methods determine OXT in presence of its degradation products. So the aim of the work was to develop and validate quantitatively selective methods for the determination of OXT in the presence of its hydrolytic and oxidative degradation products in bulk powder and in its dosage form.

The degradation products under acidic and alkaline conditions were eluted at the same R_t upon injection into HPLC which suggest they are the same compounds but hydrolysed after a longer time in case of acidic hydrolysis.

The absorption spectra of OXT, its hydrolytic and oxidative degradation products show high degree of interference as shown in Figure 2. All orders of derivative spectrophotometry failed to resolve OXT from its degradation products suggest the use of derivative ratio method. The first derivative of OXT spectrum was divided by the spectrum of 40 $\mu\text{g}/\text{mL}$ of the hydrolytic degradation products giving a good peak amplitude at 224.3 nm manipulated using scaling factor 10 and $\Delta\lambda = 8$ nm.

Meanwhile; dividing the previously prepared oxidative degradation products by the UV absorption spectrum of 40 $\mu\text{g}/\text{mL}$ of the

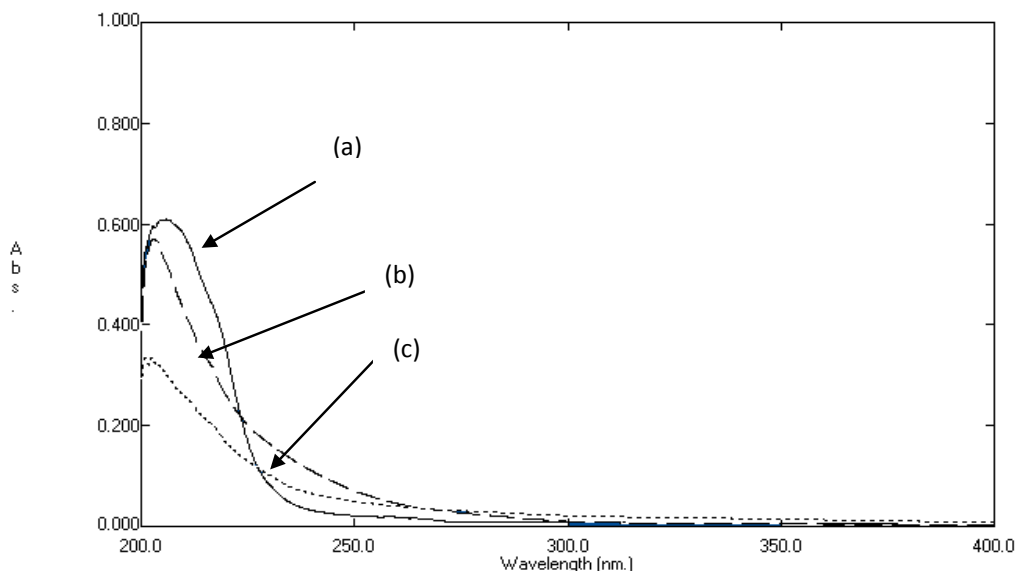


Figure 2: The absorption spectra of (a) 10 $\mu\text{g}/\text{mL}$ of OXT, (b) 40 $\mu\text{g}/\text{mL}$ of the hydrolytic degradation products (c) 4 $\mu\text{g}/\text{mL}$ of the oxidative degradation products

hydrolytic degradation products then manipulating the first derivative using the same parameters before, gives zero crossing at 224.3 nm (Figure 3) thus favour the determination of OXT at this wavelength without any interference of its hydrolytic and oxidative degradation products.

Careful choice of the divisor is mandatory; the selected divisor should compromise between minimal noise and maximum sensitivity so different concentration of the hydrolytic degradation products was tried. It was found that 40 $\mu\text{g}/\text{mL}$ of the hydrolytic degradation products gave the best results regarding selectivity although it gives lower sensitivity (Figure 3).

Ratio difference (RD) method was first developed by ELzanfaly *et al.* [9] for the simultaneous determination of binary mixtures. It

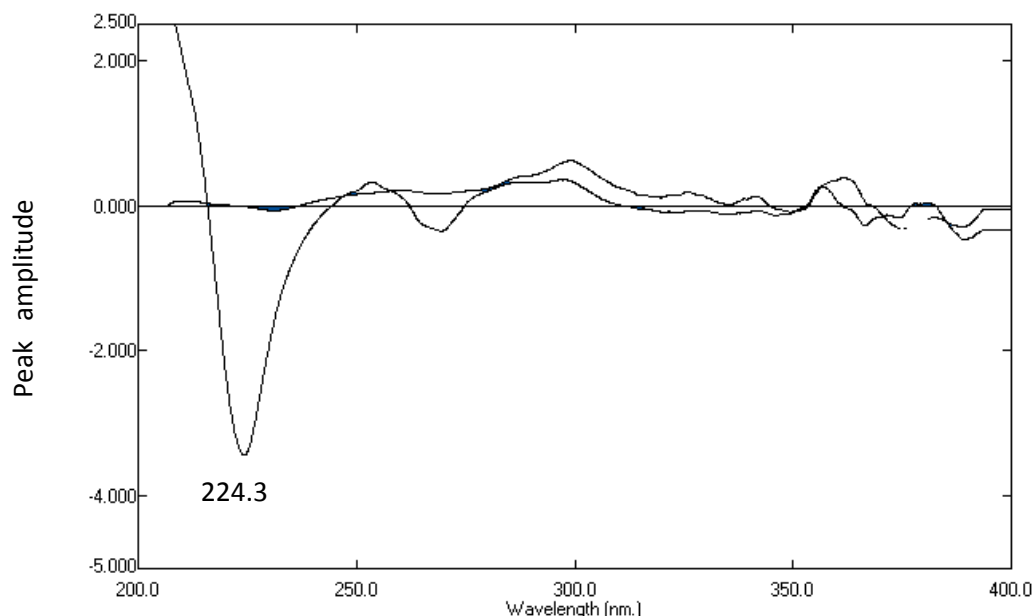


Figure 3: The first derivative of the ratio spectrum of 4 $\mu\text{g/mL}$ of the oxidative degradation products by the UV absorption spectrum of 40 $\mu\text{g/mL}$ of the hydrolytic degradation products showing zero crossing at 224.3 nm

has an advantage over the previously stated (^1DD) method in that OXT can be determined in only two steps omitting the third step of the derivatization of the ratio spectra with acceptable accuracy and selectivity. The two wavelengths at which the ratio difference of the spectrum of 4 $\mu\text{g/mL}$ of the oxidative degradation products divided by the UV absorption spectrum of 40 $\mu\text{g/mL}$ of the hydrolytic degradation products gives zero were carefully selected. It was found that 216.1 and 227.9 nm give the best results with satisfactory precision and accuracy (Figure 4).

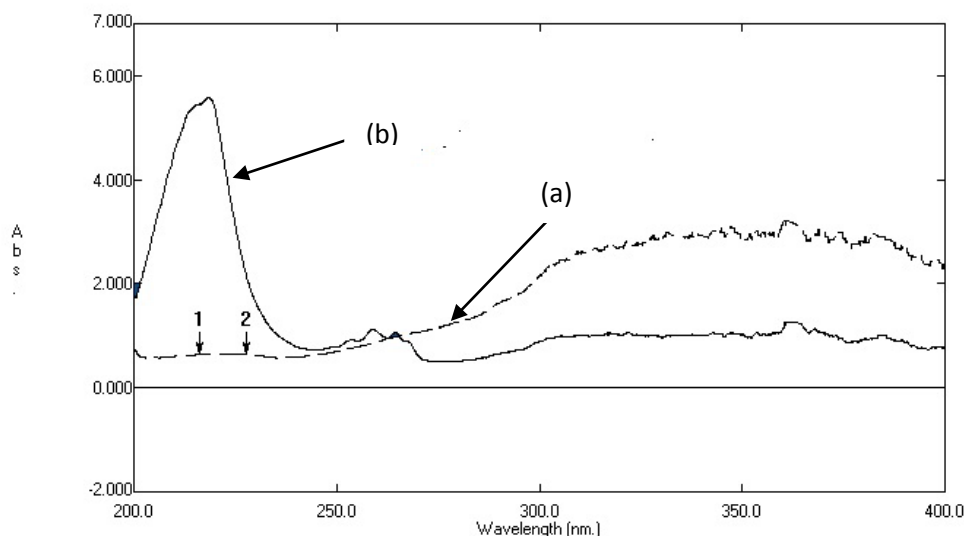


Figure 4: (a) The ratio of 4 $\mu\text{g/mL}$ of the oxidative degradation products divided by the absorption spectrum of 40 $\mu\text{g/mL}$ of the hydrolytic degradation products showing zero difference between the 216.1 nm and 227.9 nm wavelengths, (b) the ratio of 40 $\mu\text{g/mL}$ OXT divided by 40 $\mu\text{g/mL}$ of the hydrolytic degradation products

Method validation

The proposed methods were validated according to the ICH guidelines [10].

Linearity

The linearity of the proposed methods was evaluated by analysing a series of different concentrations of OXT. The linearity of the developed methods were estimated, and the linear regression data for the calibration curves showed good linearity ($r=1$) between absorbance and concentrations in case of (^1DD) method over concentrations of 8 - 40 $\mu\text{g/ml}$ and 2-40 $\mu\text{g/ml}$ in case of determination of OXT by ratio difference method.

Accuracy

Accuracy of the methods was studied by recovery experiments of three different concentrations covering the linearity range. The overall results of percent recoveries of OXT in pure drug solutions are plotted in Table 1 indicating good accuracy of the proposed methods.

Table 1: Validation parameters of the proposed methods for determination of OXT

Parameter	(¹ DD) method	Ratio difference method
Wavelength	224.3 nm	216.1 nm 227.9 nm
Linearity range	8-40 µg/mL	2- 40 µg/mL
Intercept	-0.0074	-0.009
Slope	0.0874	0.084
Correlation coefficient	0.9999	1
Accuracy (Mean ± RSD)	100.15 ± 1.54	99.44 ± 1.46
Accuracy for determination of OXT in Mucogel [®] suspension (Mean ± RSD)	99.67 ± 0.75	99.92 ± 0.83
Selectivity (Mean ± RSD)	100.5 ± 0.85	99.69 ± 1.28
Precision(RSD)		
Repeatability ^a	0.48	1.68
Intermediate precision ^b	1.18	1.43

Precision

Pure samples of OXT were analyzed over different days to obtain inter-days (intermediate precision, n=3 for each concentration) and within the same day to obtain intra-day precision (repeatability, n=3 for each concentration), then the RSDs percent values were calculated. The results of repeatability and intermediate precision experiments are shown in Table 1. The developed methods were found to be precise as the RSD% was <2% according to CDER guidance [11].

Specificity

Specificity was ascertained by analyzing different laboratory prepared mixtures of the drug and its hydrolytic and oxidative degradation products where satisfactory recovery results were obtained (Table 2).

Table 2: Determination of OXT in laboratory prepared mixtures by the proposed UV first derivative ratio method

Concentration of OXT(µg/mL)	Concentration of degradation added (µg/mL)			
	Oxidative	Hydrolytic	¹ DD	Ratio difference method
10	180	480	100.2	100.46
24	120	320	99.1	99.4
40	60	160	99.45	99.22
Mean	-	-	99.58	99.69
SD	-	-	0.56	1.27
% RSD	-	-	0.56	1.27

Analysis of OXT in Mucogel[®] suspension

The proposed methods could be successfully applied for the quantitative assay of OXT in its pharmaceutical formulation with good percentage recoveries. Results are shown in Table 1.

The results were statistical compared with the official method [2] showing no significant difference as shown in Table 3.

CONCLUSION

The proposed methods provide simple, accurate and reproducible quantitative analysis for the determination of OXT in its pure form and in its pharmaceutical suspension, without any interference from the excipients, and in the presence of its hydrolytic and oxidative degradation products. The spectrophotometric methods of analysis have the advantage of lower cost, affordability and rapidity over other quantitative analysis methods such as chromatographic methods.

Table 3: Statistical comparison of the results obtained by applying the proposed derivative ratio spectra, ratio difference spectrophotometric methods and the reported official potentiometric method for the determination of oxetacaine in its dosage form

Statistical Term	Reference method**	(¹ DD) method	Ratio Difference method
Mean	100.12	99.67	99.92
S.D	0.89	0.75	0.83
S.E	0.4	0.43	0.48
%RSD	0.89	0.75	0.83
n	5	3	3
V	0.79	0.56	0.69
t* (2.447) ^a	-	0.73	0.31
F* (9.9443) ^b	-	1.41	1.14

*Figures in parentheses are the theoretical t and F values at (p=0.05)

^a reference value for (¹DD) and ratio difference methods

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**Potentiometric method [2]

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