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SPECTROPHOTOMETRIC DETERMINATION OF PREGABALIN USING N-(1-NAPHTHYL) ETHYLENEDIAMINE, AS UV LABELING REAGENT

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ABSTRACT

A new, simple and sensitive spectrophotometric method is developed for the determination of Pregabalin (PRG).The proposed method based upon the labeling of the carboxylic moiety of PRG using N-(1-naphthyl) ethylenediamine. All parameters required for the reaction conditions are investigated. The formed derivative exhibits maximum absorbance at λ_{max} = 385 nm with a reasonable ε = 9442.34 L/mole. cm. Obedience of Beer's Law is verified with a range of 2-10 µg/mL. Validation of the procedure is evaluated according to ICH guidelines and shows good accuracy and reproducibility, with mean percentage recovery 100.1% .The Correlation Coefficient is 0.9998 (n = 5), with limit of detection 0.24 µg/mL and limit of quantification 0.74 µg/mL. The proposed method is applied successfully for the determination of PRG in its pharmaceutical formulations; the results are compared favorably with those of reference method.

KEY WORDS

Pregabalin; N-(1-Naphthyl)ethylenediamine; Spectrophotometry

1. INTRODUCTION

Pregabalin (PRG), (S)-3-(aminomethyl)-5-methylhexa-noic acid, is an antiepileptic and structurally related to the inhibitory neurotransmitter gamma aminobutyric acid (GABA) It was recently approved for adjunctive treatment of partial seizures in adults in United States and Europe and for the treatment of neuropathic pain from post therapeutic neuralgia and diabetic neuropathy[1,2]. Different methods were described for the determination of PRG in pharmaceutical formulations and biological fluids.

These methods include spectrophotometric methods[3-7], spectrofluorimetric methods[8-10], chromatographic methods[11-15] and electrophoresis[16].

N-(1-Naphthyl) ethylenediamine (NED), a component of the Griess reagent, is often used for determination of nitrite and nitrate in biological fluids[17], where a strongly colored diazo compound is formed by interaction of NED and sulfanilamide in the presence of nitrite.

NED, dihydrochloride is used for determination of sulfonamides in human urine, and pharmaceuticals[18,19]. It is used for spectrophotometric determination of flutamide[20], metronidazole and tinidazole[21], hydroxylamine and its derivatives in pharmaceuticals[22] and ceftazidime[23]. It is also

used as a labeling reagent for the liquid chromatographic determination of valproic acid[24].

This study describes a new spectrophotometric method for the determination of PRG after derivatization with NED and UV detection at 385 nm as shown in Fig. 1.

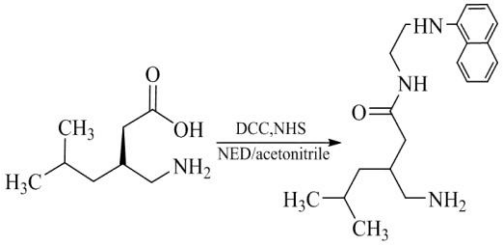

Fig. 1. Synthesis Pathway of PRG-NED.

2. EXPERIMENTAL

2.1. Apparatus

- Jasco(V-530) UV-Visible Spectrophotometer with 1 cm quartez cell.
- Digital pH meter (Consort P-300) was used for adjustment of pH.

2.2. Materials and Method

2.2.1. Reference Samples PRG pure samples were kindly supplied by (EVA Pharm Co, Egypt).The purity was provided by the company to be 99.6%.

2.2.2. Market Samples

- Lyrica capsules (Pfizer Co., Egypt) each capsule contains 75.0 mg of PRG. Batch Number: 0683062.
- Pregavalex capsules (Eva Co., Egypt) each capsule contains 75.0 mg of PRG. Batch Number: 06543.

2.2.3. Reagents

All chemicals are of analytical grade and used without further purification. N-(1-naphthyl)ethylenediamine, dicyclohexylcarbodiimide (DCC), and N-hydroxysuccinimide (NHS), sodium hydroxide, dichloromethane and acetonitrile were purchased from Merck (Darmstadt, Germany).

2.3. Preparation of Sample Solutions

- 100.0 µg/mL stock solution of PRG was prepared in sodium hydroxide solution (0.4 M).
- The working standards were prepared by serial dilution using deionized water to obtain concentrations of 2, 4, 6, 8, 10 µg/mL.

2.4. Reagents Solutions

- **N-(1-naphthyl) ethylene diamine (NED),** the reagent was freshly prepared by dissolving 40.0 mg of NED in deionized water, then sodium hydroxide solution (1 mL, 0.4 M) and 5 mL of dichloromethane were added and mixed well. The organic phase was then separated, Dried with sodium sulphate and filtered. The solvent was evaporated and the residue was dissolved in 5 mL acetonitrile.
- **N,N'-Dicyclohexylcarbodiimide (DCC),** (5.0 mg/mL) solution was prepared by dissolving 25.0 mg of DCC in 5 ml acetonitrile.
- **N-hydroxysuccinimide (NHS),** (10.0 mg/mL) solution was prepared by dissolving 50.0 mg of NHS in 5 ml acetonitrile.

2.5. Construction of Calibration Graph: Aliquots of PRG standard solution covering range of (20-100 µg/ml) were transferred to small conical flasks and treated with 200 µL acetonitrile and 200 µL NED solution. 100 µL NHS solution (10.0 mg/mL) and 100 µL DCC solutions (5.0 mg/mL) were added and mixed well.

The mixture was heated in an oven at 80 °C for one hour, and then the contents of the conical

flasks were cooled and quantitatively transferred into 10 mL volumetric flasks and completed to volume with deionized water.

A blank experiment was performed simultaneously and the absorbance was measured at 385 nm. The calibration graph was constructed by plotting the absorbance values versus drug concentration and the corresponding regression equation was derived. The overlay of the spectrum of the reaction product is shown in Fig. 2.

2.6. Application of the proposed method for analysis of PRG in dosage forms

The contents of 10 capsules were accurately Weighed and mixed well. A weighed amount of the capsules powder equivalent to 75.0 mg of PRG was transferred to small conical flask and dissolved in 20ml sodium hydroxide solution (0.4 M). The flask was sonicated for 30 minutes, the contents were filtered and transferred quantitatively to 100ml volumetric flask and completed to volume with the deionized water.

Aliquots covering the range of (2-10 µg/mL) were transferred into a series of small conical flasks, 'Procedure for calibration graph' mentioned under section (2.5) was then carried out. The nominal content of PRG within capsule is determined either from the previously plotted calibration graph or using the corresponding regression equation.

3. RESULTS AND DISCUSSION

Surveying the literature revealed that the previously studied spectrophotometric and flourimetric methods for determination of PRG based on derivatization of its amino group[3-10]. In the present study, N-(1-naphthyl) ethylenediamine chromogenic reagent (NED) is used for labeling the carboxylic group of PRG to produce a product that has a considerable UV absorbance at 385 nm. The activation of the carboxylic acid group by DCC and NHS[24,25] was carried out in acetonitrile. Formation of a stable amide bond between PRG and NED provided the sensitive detection of PRG.

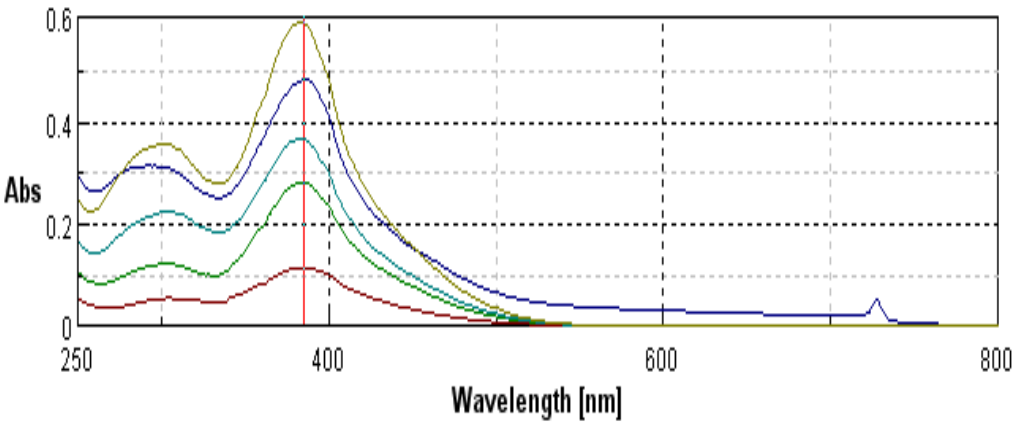

Fig. 2. The spectrum of the reaction product between PRG (2-10 µg/mL) and NED

3.1. Optimization of the experimental conditions Different experimental parameters affecting the drevatization reaction between PRG and NED were carefully studied and optimized. Such factors were changed individually, while others were kept constant. These factors include Effect of heating temperature, Effect of reaction time between PRG and NED, and Effect of concentration of NED reagent.

3.1.1. Effect of Heating Temperature The influence of heating temperature on formation of the reaction product was studied by heating the reaction mixture for 1 hour over the temperature range shown in Table 1. The maximum absorbance of the colored product was achieved after heating the reaction mixture at 80 °C for 1 hour as shown in Fig. 3.

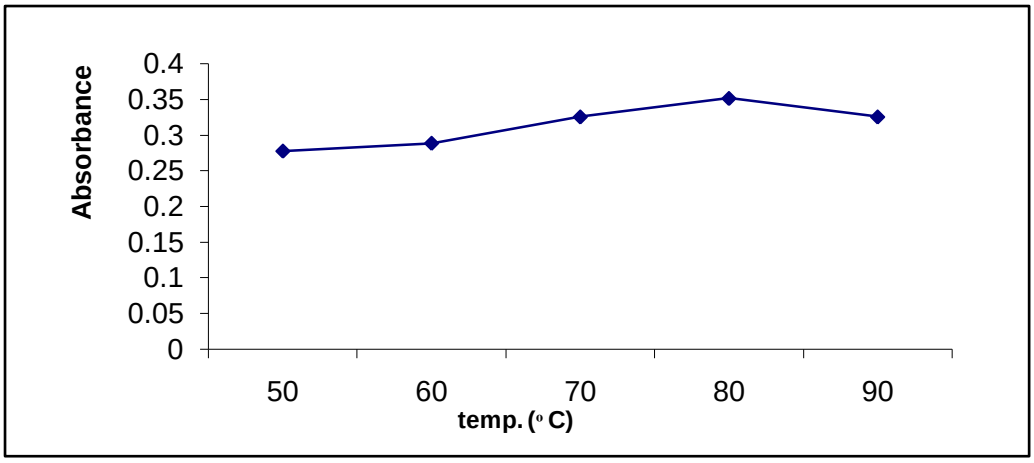

Fig.3. Effect of Heating Temperature on Formation of Reaction Product between PRG (5 µg/mL) and NED

3.1.2. Effect of Heating Time on Reaction between PRG and NED The influence of heating time on formation of the reaction product was studied by heating the reaction mixture at 80 °C for different time intervals as shown in Table 1. The maximum absorbance of the colored product was found after heating the reaction mixture for 1 hour as shown in Fig. 4.

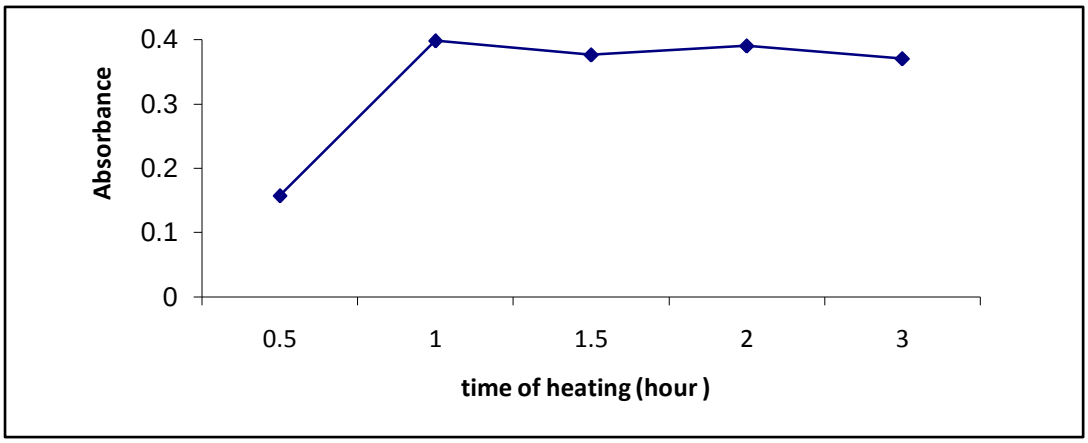

Fig. 4. Effect of Reaction Time on Formation of Reaction Product between PRG (5 µg/mL) and NED.

3.1.3. Effect of NED Reagent Concentration The effect of NED concentration on absorbance of the reaction product was studied using increasing volumes of NED reagent solution. It was found that increasing volumes of NED solution resulted in a consequent increase in absorbance of the reaction product up to 200 µL after which no further increase in absorbance was observed as shown in Table 1 and Fig. 5.

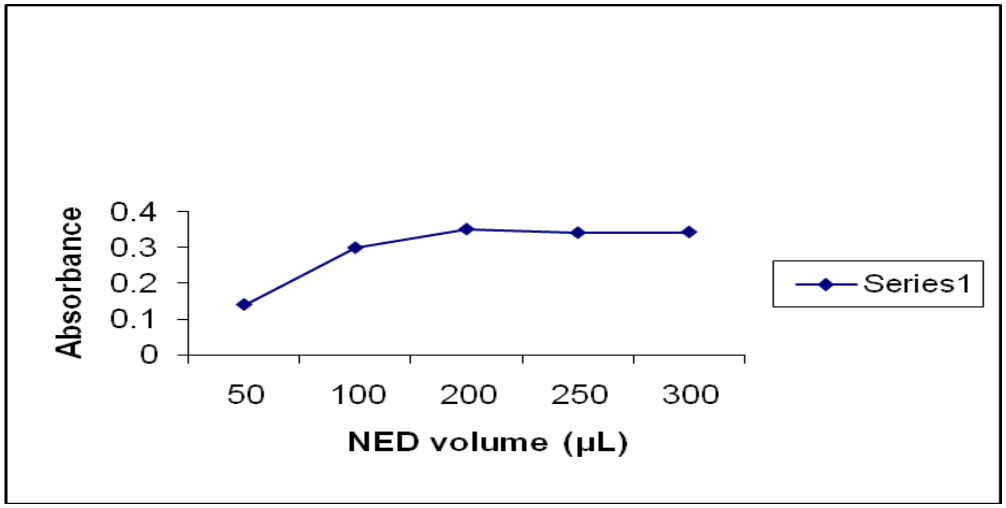

Fig. 5. Effect of NED Reagent Concentration on Formation of Reaction Product between PRG (5 µg/mL) and NED.

Table 1. Optimization of the Reaction Conditions between PRG and NED

Optimized Factor		Absorbance of the Reaction Product
Heating Temperature (°C)	50	0.278
	60	0.289
	70	0.326
	80	0.352
	90	0.326
Reaction Time between PRG and NED (minute)	30	0.157
	60	0.398
	90	0.390
	120	0.376
	180	0.370
Conc. Of NED Reagent (µL)	50	0.141
	100	0.300
	200	0.352
	250	0.342
	300	0.344

3.2. Analytical Features

After optimizing the conditions, the calibration graph was constructed by plotting the absorbance of the reaction product versus concentration of the studied drug in µg/mL. The plot was linear over the concentration range (2-10 µg/mL) with mean percentage recoveries 100.08 (n = 5).Analysis of the data gave the following regression equations:

y = 0.059x - 0.004

The absorbance of the reaction product was measured as a function of the studied drug concentration following the optimum conditions obtained from the above studied parameters to determine the quantitative range of the reaction

product at λ_{max} = 385 nm. The reaction obeys Beer's law in the range of (2-10 µg/mL), with correlation coefficient of 0.9998 and %RSD of 1.35 as shown in Fig. 6.

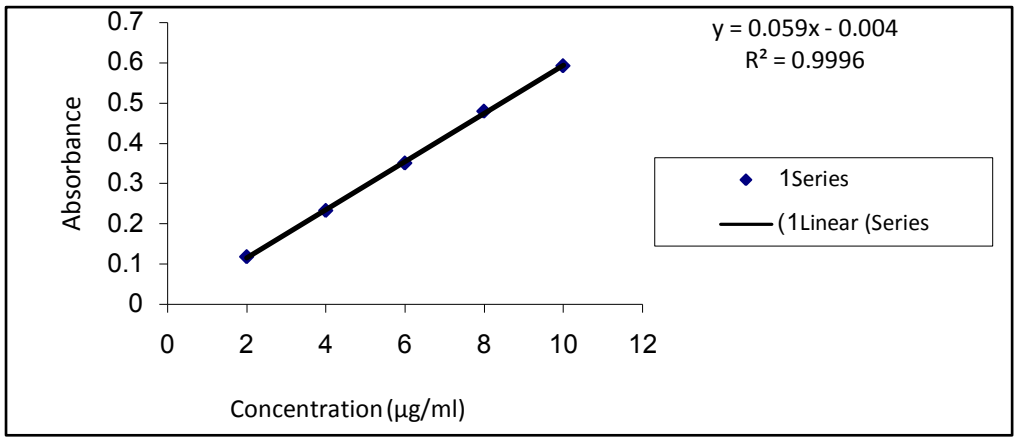

Fig. 6. Calibration Curve of the Reaction Product between PRG and NED.

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured according to ICH Q2 (R1) recommendations[26], below which the calibration graph is non linear (based on visual evaluation), while the limit of detection (LOD) was determined by evaluating the lowest concentration of the analyte that can be readily detected. The results of LOD and LOQ of the studied drug by the proposed method are abridged in Table 2

The proposed method was evaluated by studying the accuracy as percent relative error and precision as percent relative standard deviation. The results are abridged in Table 2. Statistical analysis of the results obtained by the proposed method and the comparison method for PRG[7] using Student's t-test and Variance ratio F-test shows no significant difference between the performance of our work and the comparison method regarding the accuracy and precision, respectively as shown in Table 3.

Table 2. Performance Data of the Proposed Method for Determination of PRG in Pure Form.

Parameter	PRG
Concentration range (µg/mL)	2-10 µg/mL
Regression equation	Y = 0.059x - 0.004
Correlation coefficient (r)	0.9998
Standard deviation of the residuals ($S_{y/x}$)	0.004
%Recovery	100.08
±SD	1.35
Relative standard deviation %RSD	1.35
Percentage error %Er	0.006
Limit of quantification (LOQ)	0.74 µg/mL
Limit of detection (LOD)	0.24 µg/mL

Table 3. Statistical Analysis of the Results of PRG in Pure Form by the Proposed Method, Compared with Comparison method[7].

Parameter	Proposed method			Comparison method[7]
	µg/mL Taken	µg/mL Found	%Recovery	% Recovery
	2	2.04	101.92	99.02
	4	3.96	98.96	
	6	5.93	98.80	100.79
	8	8.08	101.02	
	10	9.97	99.68	99.20
Mean %Recovery	100.08			99.67
±SD	1.35			0.974
No. of experiments	5			3
Variance	1.83			0.949
F-test	1.929 (19.25)**			
Students *t*-test	1.464 (2.45)**			

****The values in brackets are the tabulated ones at 95% confidence level.**

3.3. Validation of the proposed method

3.3.1. Linearity The proposed method was tested for linearity, specificity, accuracy and precision. Linear regression equations were obtained. The regression plots showed linear dependence of absorbance of PRG after derivatization on concentration over the range cited in Table 2. The small values of the %RSD, %Er, and residual standard deviation point out to the low scattering of the points around the calibration curve and high accuracy and precision of the proposed method.

3.3.2. Accuracy and precision The results of the intra-day and inter-day accuracy and precision of the proposed method have been summarized in Table 4. The inter-day and intra-day precisions were evaluated through replicate analysis of PRG in pure form using different concentrations (4.0, 6.0, and 8.0 µg/mL) and each concentration was measured three times a day and for three consecutive days. The precision of the proposed method was fairly high, as indicated by the low values of SD and %RSD, respectively. Also the intra-day and inter-day accuracy was proved by the low values of %Er.

Table 4. Accuracy and Precision Data of the Proposed Method for the Determination of PRG in Pure Form.

Parameter	Intra-day precision (Repeatability)			Inter-day precision (Intermediate precision)		
Concentration µg/mL	4	6	8	4	6	8
%Recovery	100.63	99.64	102.90	100.45	99.53	99.87
of pure PRG	99.37	100.47	101.02	102.21	101.37	101.98
	98.12	102.42	99.73	99.78	99.5	102.85
Mean %Recovery	99.37	100.84	101.22	100.81	100.13	101.57
±SD	1.25	1.43	1.60	1.26	1.07	1.53
%RSD	1.26	1.42	1.58	1.24	1.07	1.51
%Er	0.73	0.82	0.91	0.72	0.62	0.87

3.4. Application

3.4.1. Analysis of Pharmaceutical Formulations The proposed method was applied for determination of PRG in capsules. The results are shown in Tables 5,6,7 and 8. The results of the proposed and reference methods were

compared in accordance with the Student's t-test and variance ratio F-test[27]. There were no significant differences between the calculated and tabulated values at P 0.05, demonstrating that the proposed method is as accurate and precise as the respective reference methods.

Table 5. Accuracy and Precision Data of the Proposed Method for the Determination of PRG in Dosage Form (Lyrica 75 mg capsule).

Parameter	Intra-day precision (Repeatability)			Inter-day precision (Intermediate precision)		
Concentration µg/mL	4	6	8	4	6	8
	99.75	99.88	98.68	100.57	98.68	99.76
%Recovery	99.58	98.83	101.78	102.47	100.54	98.28
	101.86	101.55	100.38	102.38	100.8	99.62
Mean %Recovery	100.40	100.09	100.28	101.81	100.01	99.22
±SD	1.27	1.37	1.55	1.07	1.16	0.82
%RSD	1.27	1.37	1.55	1.05	1.16	0.82
%Er	0.73	0.79	0.90	0.61	0.67	0.48

Table 6. Accuracy and Precision Data of the Proposed Method for the Determination of PRG in Dosage Form (Pregavalex 75 mg capsule).

Parameter	Intra-day precision (Repeatability)			Inter-day precision (Intermediate precision)		
Concentration µg/mL	4	6	8	4	6	8
	100.58	102.67	101.84	100.97	101.05	102.69
%Recovery	98.65	100.16	102.66	98.58	100.28	100.76
	101.97	100.98	101.21	98.63	101.63	98.95
Mean %Recovery	100.4	101.27	101.9	99.40	100.99	100.8
±SD	1.67	1.28	0.73	1.37	0.68	1.87
%RSD	1.66	1.26	0.71	1.38	0.67	1.86
%Er	0.96	0.73	0.41	0.80	0.39	1.07

Table 7. Application of the Proposed Method for the Analysis of PRG in Dosage Form (Lyrica 75 mg capsule).

Parameter	Proposed method			Comparison method[7]
Concentration Taken (µg/mL)	Concentration found(µg/mL)	%Recovery	Mean %Recovery	%Recovery
4	3.962	99.05	98.70	97.89
	3.884	97.10		
	3.998	99.95		
6	5.928	98.80	98.93	98.78
	5.874	97.90		
	6.005	100.08		
8	8.082	101.03	100.80	100.18
	8.126	101.58		
	7.983	99.79		
Mean %Recovery	99.48			98.95
±SD	1.15			1.15

%RSD	1.16		1.16
%ER	0.67		0.67
No. of experiments	3		3
Variance	1.33		1.32
F- test	1.88	(19.00) *	
Student's t-test	1.36	(2.78) *	

***The values in brackets are the tabulated ones at 95% confidence level.**

Table 8. Application of the Proposed Method for the Analysis of PRG in Dosage Form (Pregavalex 75 mg capsule).

Parameter	**proposed method**			**Comparison method [7]**
Concentration taken (µg/mL)	Concentration found (µg/mL)	%Recovery	Mean %Recovery	% Recovery
4	4.005	101.37	101.28	100.48
	4.113	102.82		
	3.986	99.65		
6	5.996	99.93	100.83	99.88
	6.143	102.38		
	6.011	100.18		
8	8.163	102.04	101.15	100.22
	7.899	98.74		
	8.214	102.68		
Mean %Recovery	101.09			100.19
±SD	0.23			0.30
%RSD	0.228			0.30
%ER	0.131			0.17
No. of experiments	3			3
Variance	0.053			0.09
F- test	2.14(19.00) *			
Student's t-test	1.80(2.78)*			

***The values in brackets are the tabulated ones at 95% confidence level.**

4. CONCLUSION

The data of the results given by this proposed procedure are indicative for high sensitivity and reasonable selectivity. Furthermore, these findings are favorably comparable to other methods.

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