DOI 10.1007/s10812-019-00888-0
Journal of Applied Spectroscopy, Vol. 86, No. 4, September, 2019 (Russian Original Vol. 86, No. 4, July–August, 2019)

OPTIMIZATION AND VALIDATION OF THE SPECTROPHOTOMETRIC METHODS FOR THE ASSAY OF DEXMEDETOMIDINE HYDROCHLORIDE IN PURE AND DOSAGE FORMS

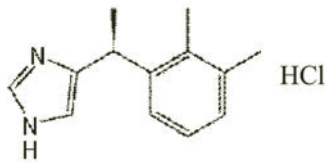
K. P. Roopa,[a] K. Basavaiah,[b] and B. K. Jayanna[c]

UDC 543.42.062:615.012.8

Four simple, precise, low-cost, sensitive, and diversely applicable UV-Vis spectrophotometric methods have been developed for the assay of dexmedetomidine hydrochloride in pure and pharmaceutical dosage forms. The methods are based on the oxidation of the drug by N-bromosuccinimide (NBS) (excess) at room temperature and estimating the amount of unconsumed NBS by amaranth dye at λ_{max} = 530 nm (method A), safranin dye at λ_{max} = 530 nm (method B), aniline blue at λ_{max} = 610 nm (method C), or rhodamine B at λ_{max} = 560 nm (method D). Regression analysis of Beer–Lambert's plots proves excellent correlation in the concentration ranges 2–9, 4–11, 2–10, and 1.2–3.5 μg/mL for methods A, B, C, and D, respectively. The apparent molar absorptivity, Sandell's sensitivity, and detection and quantification limits are calculated. The proposed methods can be applied to drug formulation and recommended for routine analysis in quality control laboratories. Statistical comparison of the results with the reference method shows excellent agreement.

Keywords: *dexmedetomidine hydrochloride, N-bromosuccinimide, spectrophotometry, pharmaceutical preparations.*

Introduction. Dexmedetomidine hydrochloride [1] (DEX.HCl), chemically described as (+)-4-(S)-[1-(2, 3-dimethylphenyl)-ethyl]-1H-imidazole monohydrochloride,



is a potent and highly selective α_2-adrenergic receptor agonist widely used for the sedation of initially incubated and mechanically ventilated patients in intense care units (ICU). DEX.HCl also offers good perioperative hemodynamic stability, an intraoperative anesthetic-sparing effect, and has sedative, anxiolytic, and analgesic effects [2–7]. In addition to this, it also provides cardioprotection in coronary bypass graft surgeries [8], reduces the renal injury in the rat kidney [9], and balances pro- and antiapoprotic proteins [10]. It plays a major role in cellular plasticity and survival in rats [11]. DEX.HCl API is official in USP [12].

A literature survey reveals a few analytical methods for the estimation of DEX.HCl in biological fluids and dosage forms: HPLC [13–17], LC-MS [18–20], UV [21], and spectrophotometry [22]. However, the reported methods are time-consuming, tedious, and require expensive analytical instruments. Spectrophotometric methods are the most convenient techniques because of their inherent simplicity, high sensitivity, low cost, and wide availability in quality control laboratories. Therefore, the development and validation of new spectrophotometric methods for the determination of DEX.HCl are important.

The problem of the spectrophotometric determination of organic compounds in pharmacology was considered in [23–26]. Unfortunately, there are no reports on the application of spectrophotometric methods with NBS. In this paper, we describe simple, sensitive, accurate, precise, and elegant spectrophotometric methods for the determination of DEX.HCl in

[a]Sapthagiri College of Engineering, Department of Chemistry, Bangalore, India; email: roopakp@sapthagiri.edu.in, roopakp51@gmail.com; [b]University of Mysore, Department of Studies in Chemistry, Manasagangothri Mysore; [c]B.N.M. Institute of Technology, Department of Chemistry, Bangalore, India. Abstract of article is published in Zhurnal Prikladnoi Spektroskopii, Vol. 86, No. 4, p. 670, July–August, 2019.

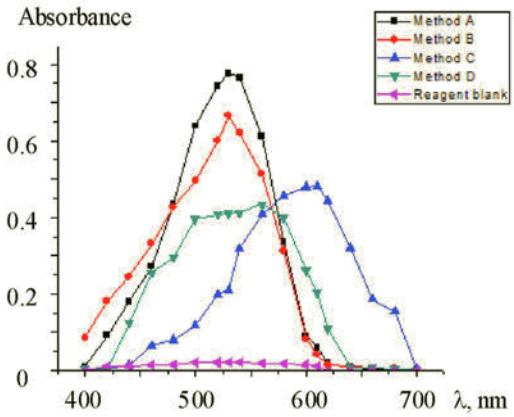


Fig. 1. Absorption spectra of DEX.HCl with amaranth dye (8 μg/mL) (method A), safranin dye (9.0 μg/mL) (method B), aniline blue (6 μg/mL) (method C), rhodamine B (2.4 μg/mL) (method D) against reagent blank.

bulk and pharmaceutical dosage forms. These methods are based on the oxidation of DEX.HCl with an excess of NBS, and unconsumed NBS is determined by its reaction with four dyes such as amaranth, safranin, aniline blue, and rhodamine B. The methods are more sensitive than the existing ones and free from the impact of such experimental variables as heating or the extraction step. The methods rely on the use of simple, inexpensive chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC. Statistical analysis of the results indicates that the method yields reproducible values. Hence the proposed methods are validated as per ICH guidelines and can be successfully applied for routine drug determination in pharmaceutical formulations.

Experiment. A BL 198 Bio spectrophotometer (UV-Vis) with 1.0 cm matched cells was used for spectral measurements. All reagents were of analytical grade, and double distilled water was used throughout the experiment. Dexmedetomidine hydrochloride was obtained as a gift from Mylon, India; amaranth, safranin, aniline blue, and rhodamine B from S.D. Fine Chemicals PVT., Ltd., Mumbai, India, were prepared in the required amount of distilled water. NBS was from Merck, Germany; H_2SO_4 and HCl were from Ranbaxy Fine Chemicals, India.

A stock solution of dexmedetomidine hydrochloride (100 μg/mL) was prepared by dissolving 10 mg of the drug in water and diluted in a 100-mL volumetric flask. The solution was further diluted quantitatively according to their linearity range.

NBS was prepared by dissolving 0.02 g of the chemical in water with the aid of heat and diluting to 100 mL and standardized [27]. The NBS solution was kept in a refrigerator when not used.

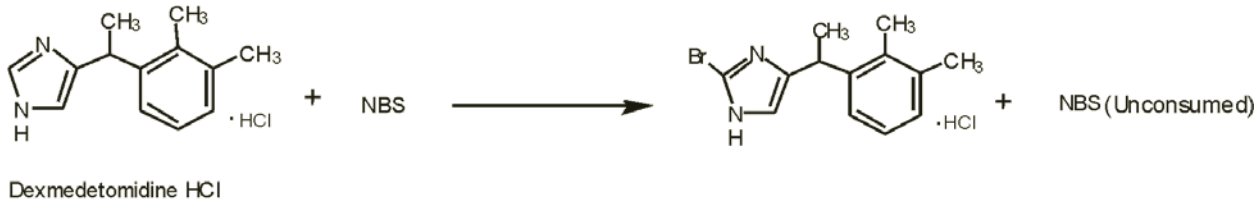
For the analysis of the injection, the required amount of the drug (10 μg of DEX.HCl) was transferred to a 100-mL volumetric flask and diluted with distilled water. An aliquot of the solution was analyzed as described under the general procedure.

Different aliquots of standard DEX.HCl solution ranging from 2–9, 4–11, 2–10, and 1.2–3.5 μg/mL were transferred into a series of 10 mL calibrated flasks for methods A, B, C, and D, respectively. To each flask containing the drug, in the order mentioned above, 1.0 mL of 0.02% NBS, after 5 min 0.4 mL of 0.1% amaranth dye (method A), 0.4 mL of 0.03% safranin dye (method B), 0.6 mL of 0.2 M H_2SO_4, and 0.9 mL of 0.02% aniline blue dye (method C), and 0.5 mL of 1 M HCl, and 1.1 mL of 0.01% rhodamine B (method D) were added. The contents were mixed well, the volume was made up to the mark with water, and the absorbance of each solution was measured at 530, 530, 610, and 560 nm against a reagent blank, similarly prepared in the absence of the drug.

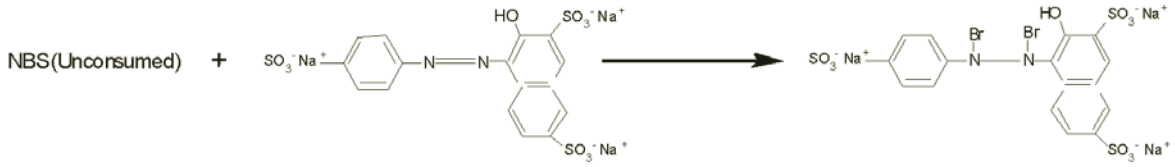
Results and Discussion. The absorption spectra of the reaction products of dexmedetomidine hydrochloride and the corresponding reagent blank for methods A, B, C, and D are shown in Fig. 1. Beer's law was obeyed in the concentration range 2–9, 4–11, 2–10, and 1.2–3.5 μg/mL for methods A, B, C, and D, respectively. The curves were found to be linear with different slopes and characterized by high correlation coefficients in all cases.

The developed spectrophotometric methods are based on the redox reaction between the drug, dye, and NBS (methods A and B), or drug, dye, and NBS in an acidic medium (methods C and D) at room temperature, respectively. In all the developed methods, NBS acts as an oxidizing agent. The proposed spectrophotometric methods are based on the reaction

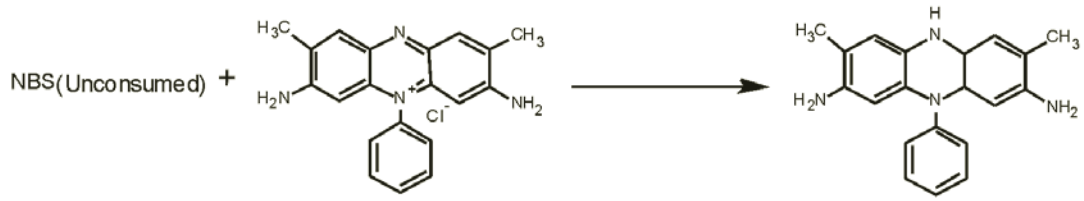
between DEX.HCl and the measured excess of NBS and the subsequent determination of the latter by its reaction with a fixed amount of amaranth, safranin, aniline blue, or rhodamine B in an acidic medium with measuring the absorbance at 530, 530, 610, and 560 nm. These methods use the bleaching action of NBS on the dyes (the decolorization is caused by the oxidative destruction of the dyes). A fixed concentration of the dye was added to the decreasing concentration of NBS. Increasing the dye concentration after that is made proportional to the increasing concentration of the drug. The suggested reaction sequence is shown in Scheme 1.



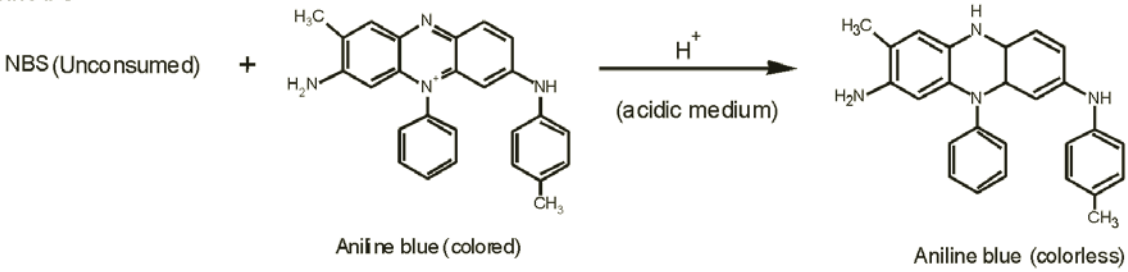
Method A



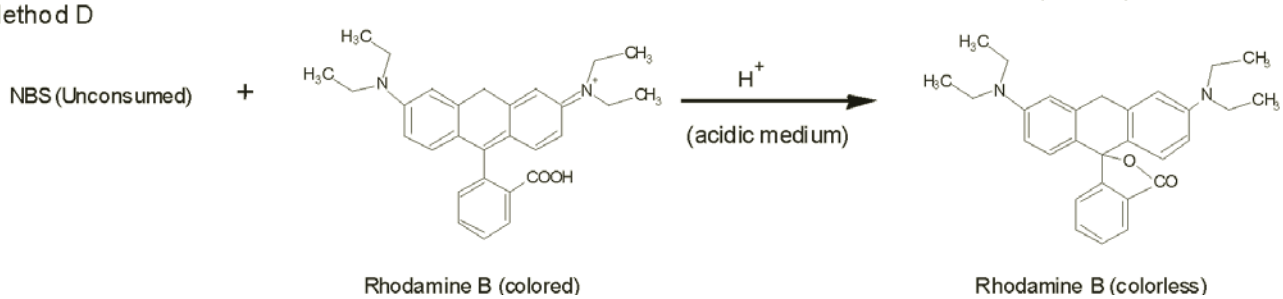
Method B



Method C



Method D



Scheme 1.

TABLE 1. Optical Characteristics and Regression Parameters of the Proposed Methods

Parameter	Method A	Method B	Method C	Method D
Color	pink	pink	blue	pink
λ_{max}, nm	530	530	610	560
Beer's law limit, μg/mL	2–9	4–11	2–10	1.2–3.6
Molar absorptivity, $L \times mol^{-1} \times cm^{-1}$	1.7071×10^4	1.1043×10^4	1.3322×10^4	3.7034×10^4
Sandell's sensitivity, $\mu g/cm^2$	0.01173	0.018136	0.015033	5.4079×10^{-4}
LOD, μg/mL	0.0567	0.2274	0.0475	4.2556×10^{-4}
LOQ, μg/mL	0.17182	0.68407	0.14402	1.2896×10^{-3}
Regression equation $Y = BX+A$				
Slope B	0.1125	0.1118	0.1097	2.90135
Intercept A	–0.115	–0.38457	–0.2003	0.98198
Correlation coefficient r	0.9974	0.9901	0.9981	0.9941
Relative standard deviation*	0.014	0.018	0.014	0.018

Note. X is the concentration of the measured solution (μg/mL) and Y is the unit for absorbance.

*Average of five determinations (concentrations of 3, 5, and 7 μg/mL (method A), 5, 7, and 9 μg/mL (method B), 4, 6, and 8 μg/mL (method C), and 1.6, 2.4, and 3.2 μg/mL (method D) for DEX.HCl, respectively.

N-Bromosuccinimide is used widely as an oxidizing agent for organic compounds. NBS has the ability to oxidize the drug and dyes; 0.02% of NBS was found to be the optimal value for the drug oxidation. The order of addition of the reagents plays a major role in the drug formulation. The drug solution added before the addition of the dyes showed the maximum absorbance, and this order of addition was selected for all further determinations.

The reaction was carried out at room temperature (25 ± 30°C). Satisfactory maximum color intensity and reproducible λ_{max} was obtained at room temperature. It was found that 10 min was optimum for the drug oxidation after the addition of the dyes; 2–5 min was required for bleaching. The colored products were stable for more than 24, 2, 2, and 12 h for methods A, B, C, and D, respectively.

The validity of the proposed methods was tested regarding linearity, range, limits of detection, limits of quantification, accuracy, and precision according to the ICH guidelines [28]. Beer's law range, molar absorptivities and Sandell's sensitivities, regression equation, and correlation coefficients were evaluated and given in Table 1. A linear relationship was found within the ranges 2–9, 4–11, 2–10, and 1.2–3.5 μg/mL for methods A, B, C, and D, respectively. The proposed methods showed excellent linearity for the determination of the drug with high correlation coefficients in the range 0.9901–0.9981. High molar absorptivity in the range 10^3–10^4 and low Sandell's sensitivity values (0.011–5.4×10^{-4}) showed that the methods were sensitive. Regression analysis of the Beer's law plots revealed a good correlation. The calibration graphs showed a negligible intercept as described by the regression equation obtained by the least square method. The limits of detection (LOD) and the limits of quantification (LOQ) were calculated as LOD = $3.3\sigma/S$, LOQ = $10\sigma/S$, where σ was the standard deviation of reagent blank determination, and S was the slope of the calibration curve. Such values confirm the excellent sensitivity of the proposed methods. Beer's law curves of DEX.HCl with dyes for methods A, B, C, D are shown in Fig. 2.

The effects of common excipients used in the pharmaceutical preparation were studied by analyzing synthetic sample solutions containing the quantity of drug as mentioned in Table 2 in the presence of a 100-fold concentration of each excipient. The tolerance limit was defined as the concentration giving an error of ±3.0% in the determination of the drug. Common excipients such as dextrose, lactose, talc, and starch had no influence on the analysis.

The precision of the methods was calculated in terms of intermediate precision by taking five replicate measurements (intraday and interday). Intraday precision was evaluated by measuring five independent samples at three different concentration levels 3, 5, 7; 5, 7, 9; 4, 6, 8, and 1.6, 2.4, 3.2 μg/mL for methods A, B, C, and D, respectively. Similarly, interday precision at the same concentration level was repeated for five consecutive days (Table 3). The percentage relative

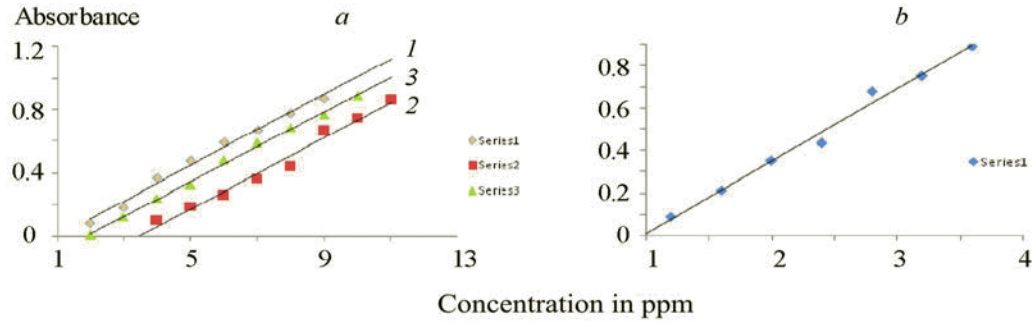


Fig. 2. Beer's law curves of DEX.HCl: a, with amaranth (1), safranin (2), and aniline blue (3) for methods A, B, and C; b, with rhodamine B (method D).

TABLE 2. Recovery (± % RSD[a]) of the Drug from the Solution with a 100-fold Excess of Various Additives Used as Excipients

Excipients	Method A[b]	Method B[c]	Method C[d]	Method D[e]
Lactose	99.8 ± 0.2	99.8 ± 0.2	99.9 ± 0.2	99.7 ± 0.3
Sucrose	98.7 ± 0.4	98.7 ± 0.4	99.7 ± 0.3	99.6 ± 0.2
Dextrose	100.0 ± 0.1	100.0 ± 0.1	100.0 ± 0.1	100.0 ± 0.2
Talc	99.7 ± 0.3	99.7 ± 0.3	99.9 ± 0.3	98.9 ± 0.2
Starch	99.8 ± 0.1	99.9 ± 0.2	100.01 ± 0.1	99.7 ± 0.2

[a]Mean ± % RSD, $n = 3$, mean of three determinations.
[b]Concentration of DEX.HCl used 5 µg/mL (method A).
[c]Concentration of DEX.HCl used 7 µg/mL (method B).
[d]Concentration of DEX.HCl used 6 µg/mL (method C).
[e]Concentration of DEX.HCl used 2.4 µg/mL (method D).

TABLE 3. Intraday and Interday Precision Data of the Determination of DEX.HCl

Formulation	Intraday			Interday	
	Amount taken (µg/mL)	Amount found (µg/mL)	% Recovery ± % RSD[a]	Amount found (µg/mL)	% Recovery ± % RSD[b]
DEX.HCl (A)	3.0	3.10	100.3 ± 2.15	2.99	96.65 ± 1.98
	5.0	5.02	100.4 ± 1.34	4.97	99.40 ± 1.25
	7.0	6.99	99.85 ± 0.99	7.01	100.1 ± 1.02
DEX.HCl (B)	5.0	4.98	99.60 ± 1.39	5.01	100.2 ± 1.29
	7.0	7.01	100.1 ± 2.90	6.99	99.85 ± 2.82
	9.0	8.99	99.80 ± 1.25	8.94	99.33 ± 1.21
DEX.HCl (C)	4.0	3.99	99.75 ± 2.23	3.98	99.50 ± 2.18
	6.0	6.02	100.3 ± 0.84	5.97	99.50 ± 0.75
	8.0	7.96	99.50 ± 1.42	7.98	99.75 ± 1.40
DEX.HCl (D)	1.6	1.601	100.0 ± 1.69	1.59	99.37 ± 1.56
	2.4	2.39	99.58 ± 2.60	2.40	100.0 ± 2.70
	3.2	3.18	99.37 ±2.71	3.19	99.68 ± 2.69

[a]Mean value of five determinations,
[b]Mean of five determinations performed over a period of five days.

TABLE 4. Analysis of Drugs in Pharmaceutical Formulations

Method	Drug formulations	Label claimed	% Recovery ± SD	
			Proposed method [a]	Reference method (UV)
A	[b]Dexem, inj	100 µg/1 mL	99.94 ± 0.50 t = 0.77 F = 2.01	99.67 ± 0.71
B	Dexem, inj	100 µg/1 mL	99.27 ± 0.82 t = 0.40 F = 1.13	98.99 ± 0.77
C	[c]Dextomid	200 µg/2 mL	199.24 ± 0.46 t = 0.36 F = 1.39	199.15 ± 0.39
D	Dextomid	200 µg/2 mL	200.01 ± 0.7 t = 0.25 F = 1.15	199.91 ± 0.65

[a]Mean of five determinations ± standard deviation. n = 5; the t- and F-values obtained after comparison to the reference methods, which have the following theoretical values at 95% confidence limit t = 2.44 and F = 5.05. After adding the pure drug to the fixed concentration of preanalyzed pharmaceutical formulations.

[b]DEX.HCl equivalent to 100 µg/1 mL (Themis Medicare Ltd., India) for methods A & B.

[c]DEX.HCl equivalent to 200 µg/2 mL (Neon Laboratories Ltd., India) for methods C & D.

TABLE 5. Results of the Recovery Study via the Standard Addition Method

Method	Formulation	Amount of drug taken in inj, µg/mL	Amount of pure drug added, µg/mL	[*]Total found, µg/mL	% Recovered ± % RSD
A	Dexem, inj (100 µg/mL)	3.0	1.0	4.01	100.25 ± 1.19
		3.0	3.0	5.99	99.83 ± 1.03
		3.0	5.0	7.98	99.75 ± 2.01
B	Dexem, inj (100 µg/mL)	2.0	4.0	5.99	99.83 ± 1.27
		2.0	6.0	7.78	97.25 ± 0.74
		2.0	8.0	9.91	99.1 ± 0.57
C	Dextomid (200 µg/2 mL)	2.0	3.0	4.97	99.40 ± 2.0
		2.0	5.0	6.86	99.80 ± 1.1
		2.0	7.0	8.97	99.66 ± 1.7
D	Dextomid (200 µg/2 mL)	1.0	1.0	1.98	99.0 ± 1.0
		1.0	1.8	2.75	98.21 ± 2.5
		1.0	2.6	3.56	98.88 ± 2.1

[*]Mean value of five determinations.

standard deviation values were ≤ 2% (intraday) and ≤3% (interday), indicating good precision of the methods. The available pharmaceutical dosage forms of the investigated drug were analyzed by the proposed methods.

The proposed methods were applied to the quantification of DEX.HCl in formulations. The results in Table 4 showed that the methods are successful for the determination of DEX.HCl, and the excipients in the dosage form do not interfere. The

results obtained from the assay of DEX.HCl by the proposed methods and the reference method [21] are presented in Table 4. The results agree well with the label claim and are also in agreement with the results obtained by the reference method. When the results were statistically compared with those of the reference method by applying Student's *t*-test for accuracy and *F*-test for precision, the calculated *t*- and *F*-values at 95% confidence level did not exceed the tabulated values ($t = 2.44$, $F = 5.05$), respectively, for five degrees of freedom. Hence, no significant difference existed between the proposed methods and the reference method with respect to accuracy and precision.

The reliability and accuracy of the proposed methods were further ascertained through recovery studies using the standard addition method (adding different amounts of the standard drug to the pre-analyzed dosage forms so that the cumulative amount after adding the drug did not exceed their linearity range). The recovery of the pure drug added was quantitative, and the co-formulated substances starch, talc, dextrose, and lactose did not interfere in the determination. The results of the recovery study are compiled in Table 5.

Conclusions. The proposed spectrophotometric methods are very simple, rapid, sensitive, and reproducible for the determination of DEX.HCl in pharmaceutical forms. The methods do not suffer from the instability of colors as the bleaching of dye is involved. The time required for the entire analysis is only 15–20 min. The proposed methods have comparable analytical performances and are devoid of any potential interference. The accuracy, precision, and cost-effectiveness of the methods suggest their potential application in quality control laboratories where sophisticated instruments are not available. Therefore, the proposed methods can be recommended for the routine analysis of DEX.HCl in quality control laboratories.

REFERENCES

1. J. MaryadeleJ, B. K. Cherie, and J. R. Kristin, *The Merck Index of an Encyclopedia of Chemicals, Drugs and Biologicals*, 14th ed., Merck Research Laboratory Publication, USA (2006).
2. D. S. Carollo, B. D. Nossaman, and U. Ramadhyani, *Curr. Opin. Anesthesiol*., **21**, 457–461 (2008).
3. A. Arcangeli, C. D'Alo, and R. Gaspari, *Curr. Drug. Targets*., **10**, 687–695 (2009).
4. P. E. Tanskanen, J. V. Kytta, T. T. Randell, and R. E. Aantaa, *Br. J. Anaesth.*, **97**, 658–665 (2006).
5. H. Yagmurdur, N. Ozcan, F. Dokumaci, K. Kilinic, and F. Yimaz Hulya Basar, *J. Hand. Surgery.*, **33**, 941–947 (2008).
6. Uysal Hale Yarkan, Cuzdan Saut Seat, Karyan Oguz, Basar Hulya, and Fidanc Vidan, *J. Craniofacial Surg.*, **23**, 1287–1291 (2012).
7. Y. Jang, M. Y. Yeom, E. S. Kang, J. W. Kang, and H. K. Song, *Int. J. Med. Sci*., **11**, 226–233 (2014).
8. H. Okada, T. Kurita, K. Mochizuki, and S. Morita Sato, *Resuscitation*., **74**, 538–545 (2007).
9. H. Kocoglu, H. Ozturk, H. Ozturk, F. Yilmaz, and N. Gulcu, *Ren. Fail*., **31**, 70–74 (2009).
10. K. Engelhard, C. Werner, E. Eberspacher, M. Bachl, M. Blobner, E. Hildt, P. Hutzler, and E. Kochs, *Anesth. Analg*., **96**, 524–531 (2003).
11. S. Dahmani, D. Rouelle, P. Gressens, and J. Mantz, *Anesthesiology*, **103**, 969–977 (2005).
12. *US Pharmacopoeia NF, The Standard of Quality, The Official Compendia of Standard*, Asian ed., **37**, 2556–2558 (2009).
13. Wenjing Li, Zunjian Zhang, Liliwu, Shudan Feng, and Yun Chen, *J. Pharm. Biomed. Anal*., **50**, 897–904 (2009).
14. Y. H. Hui, K.C. Marsh, and S. Menacherry, *J. Chromatogr. A*, **762**, 281–291 (1997).
15. Z. Cuia, L. Chowa, R. Rodrigo, O. Olutoyeb, and A. Olutoye, *J. Chromatogr. B: Biomed. Appl*., **13**, 961–967 (2014).
16. I. James, S. Felice, S. Heng, and F. Athena, *J. Chromatogr. B: Biomed. Appl*., **25**, 195–196 (2007).
17. C. Ji Qin, Y. Zhou Julie, R. Gonzals John, M. Gage Eric, and A. El-Shourbagy Tawakol, *Rapid Commun. Mass. Spectrom*., **18**, 1753–1760 (2004).
18. C. R. Preslaski, S. W. Mueller, M. F. Wempe, and R. Maclaren, *Am. J. Health-System Pharm*., **70**, 1336–1341 (2013).
19. R. R. Riker, Y. Shehabi, P. M. Bokesch, D. Ceraso, W. Wisemandle, F. Koura, P. Whitten, B. D. Margolis, D. W. Byrne, and M. G. Rocha, *J. Am. Med. Assoc*., **301**, 489–499 (2009).
20. A. Michael Frolich, A. Alireza, K. Charles, Jeevan Prasain, and B. Stephen, *J. Clin. Anesth.,* **23**, 218–223 (2011).
21. D. Khushbu Soyanatr, B. Darshil Shah, G. Dilip, and Maheshwari, *Am. J. Pharm. Health Res*., **3**, 101–107 (2015).
22. K. Harinadha Baba, C. Rambabu, K. Varaprasada Rao, Riyaz Ahmed Khan, and K. V. S. Prasada Rao, *Chem. Sci. Trans*., **4**, 270–274 (2015).
23. V. Hanci, B. Erol, S. Bektas¸ G. Mungan, S. Yurtlu, H. Tokgoz, M. Can, and I. Ozkocak Turan, *Urol. Int.*, **84**, 105–111 (2010).
24. S. Schaak, D. Cussac, C. Cayla, J. C. Devedjian, R. Guyot, H. Paris, and C. Denis, *Gut*, **47**, 242–250 (2000).

25. Daging Ma, M. Hossain, N. Rajakumaraswamy, and M. Arshad, *Eur. J. Pharm*., **502**, 87–97 (2004).
26. P. Kortesuo, M. Ahola, M. Kangas, T. Leino, S. Laakso, L. Vuorilehto, A. Yli-Urpo, J. Kiesvaara, and M. Marvola, *J. Control. Release*, **76**, 227–238 (2001).
27. A. Berka, J. Vulterin, and J. Zyoka, *Newer Redox Titrants*, Pergamon Press, New York (1965).
28. *ICH Steering Committee, ICH harmonized tripartite guideline, Validation of analytical procedures, text and methodology Q2 (R1).* In: Proceedings of the International Conference on Harmonization of Technical requirements for Registration of Pharmaceuticals for Human Use, London, UK, November (1996).