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Short communication

# A simple spectrophotometric method for the determination of β-blockers in dosage forms

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**Abstract**

A simple, extraction-free spectrophotometric method is proposed for the analysis of some β-blockers, namely atenolol, timolol and nadolol. The method is based on the interaction of the drugs in chloroform with 0.1% chloroformic solutions of acidic sulphophthalein dyes to form stable, yellow-coloured, ion-pair complexes peaking at 415 nm. The dyes used were bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP). Under the optimum conditions, the three drugs could be assayed in the concentration range 1–10 μg ml$^{-1}$ with correlation coefficient ($n = 5$) more than 0.999 in all cases. The stoichiometry of the reaction was found to be 1:1 in all cases and the conditional stability constant ($K_F$) of the complexes have been calculated. The free energy changes ($\Delta G$) were determined for all complexes formed. The interference likely to be introduced from co-formulated drugs was studied and their tolerance limits were determined. The proposed method was then applied to dosage-forms the percentage recoveries ranges from 99.12–100.95, and the results obtained were compared favorably with those given with the official methods.


*Keywords:* Atenolol; Timolol; Nadolol; β-Blockers; Sulphophthalein dyes; Dosage forms

## 1. Introduction

β-Blockers constitute one of the most frequently prescribed groups of cardiovascular drugs. They are competitive antagonists at β-adrenergic receptor sites and are used in the management of cardiovascular disorders, such as hypertension, angina pectoris, cardiac arrhythmias and myocardial infarction. Some β-blockers are used as eye drops to control intra-ocular pressure in glucoma and acular hypertension [1].

Reviewing the literature revealed that several methods have reported for the analysis of this group, whether in pharmaceutical preparations or in biological fluids. These methods include: spectrophotometry [2–4], NMR spectroscopy [5,6], fluorometry [7,8], potentiometry [9], TLC [10,11], GC [12,13], HPLC [14–18], capillary electrophoresis [19,20] and electrocapillary chromatography [21–23]. These methods are, either not sufficiently sensitive [2–9] or tedious and require highly sophisticated instrumentation [12–23]. In this piece of work, a very simple, extraction-free method is proposed for the determination of the studied compounds based on their interaction with sulphophthalein dyes in non-polar solvents.

The ion pair extraction technique has some difficulties and inaccuracies arising from incomplete extraction or the formation of emulsions between the solvent and the basic compound-containing solution. In response to the problem resulting from extraction of the ion pair few articles were published for the analysis of pharmaceutical compounds through ion pair formation without extraction [27,28].

This paper describes for the first time the application of acidic dyes to the spectrophotometric determination of β-blockers. The formed ion pair require no extraction step and are measured directly in chloroform. The proposed method was applied successfully to the determination of β-blockers either per-se or in pharmaceutical preparations with good

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accuracy and precision. Interference from some commonly co-formulated drugs was also studied.

## 2. Experimental

### 2.1. Apparatus

Beckman Du-65 Spectrophotometer (Fullerton, USA) with 1 cm quartz cells.

### 2.2. Materials and reagents

- Atenolol, timolol and nadolol were kindly provided by various manufacturers, and used as received. Dosage forms containing these drugs were obtained from commercial sources in the local market.
- Bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP) (Merck, Darmstadt, Germany) 0.1% (w/v) solutions were prepared in $CHCl_3$.
- Chloroform, Winlab, UK.
- Standard solutions of atenolol, timolol and nadolol. 0.1% (w/v) solutions were prepared in $CHCl_3$.
- These solutions are stable for at least 1 week if kept in the refrigerator.

### 2.3. Procedures

#### 2.3.1. Recommended analytical procedure

Transfer aliquot volumes of the standard solutions of the drugs into a series of 10 ml measuring flasks so that the final concentration is in the range of 1–10 $\mu g\,ml^{-1}$. Add 1 ml of the dye solution, mix and complete to volume with $CHCl_3$. Measure the absorbance of the resulting solutions at 415 nm against the corresponding blank solutions. Plot the absorbance versus the final concentration to get the calibration graph. Alternatively, derive the corresponding regression equation.

#### 2.3.2. Procedure for the tablets

Weigh and pulverize 10 tablets. Transfer a weighed amount of the powder equivalent to 100 mg of the drug into a small flask. Extract with $3 \times 20$ ml of $CHCl_3$ and filter into a 100 ml volumetric flask. Wash the residue and filter with few ml of $CHCl_3$ and pass the washings to the same flask. Complete to the mark with the same solvent. Transfer suitable aliquots of the solution into a 10 ml volumetric flask then proceed as described under Section 2.3.1. Determine the content of the drug either from the calibration graph or using the corresponding regression equation.

#### 2.3.3. Procedure for timolol in the eye drops

Mix the contents of five bottles. Transfer an accurately measured volume of the mixed solution, equivalent to 100 mg of timolol into a 100 ml separating funnel. Extract with $CHCl_3$ ($3 \times 20$ ml). Collect the chloroform extract in a 100 ml volumetric flask and complete to volume using the same solvent. Proceed as described under Section 2.3.1.

## 3. Results and discussion

Most of the β-blockers are weakly absorbing light in the UV region. The A%, 1 cm of atenolol at 274 nm is 48, and that of nadolol at 278 nm is 38. As a consequence, poor sensitivity will be achieved by conventional UV spectrophotometric methods. The structural formulae of all β-blockers feature secondary amino group. This structure suggests the use of acidic dyes as chromogenic reagents. The studied compounds

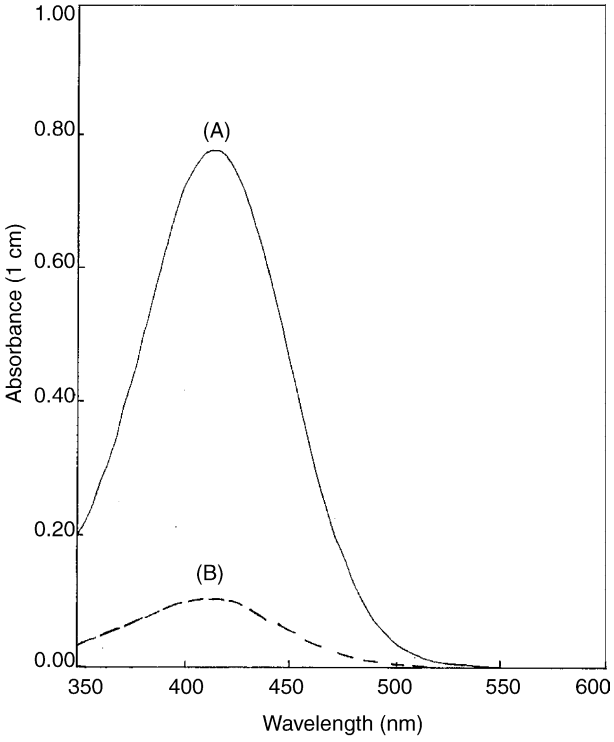



Fig. 1. Absorption spectra of the reaction product of atenolol ($7\,\mu g\,ml^{-1}$) and bromophenol blue. (A) Reaction product; (B) bromophenol blue.

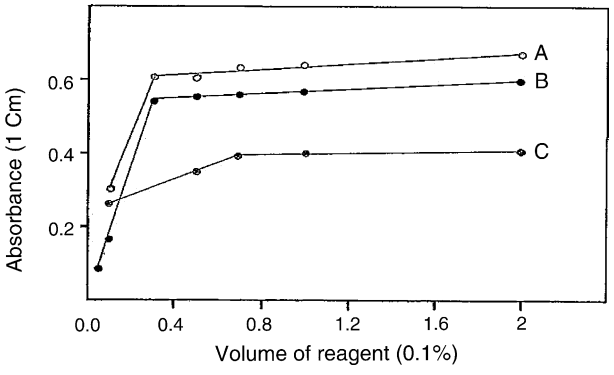
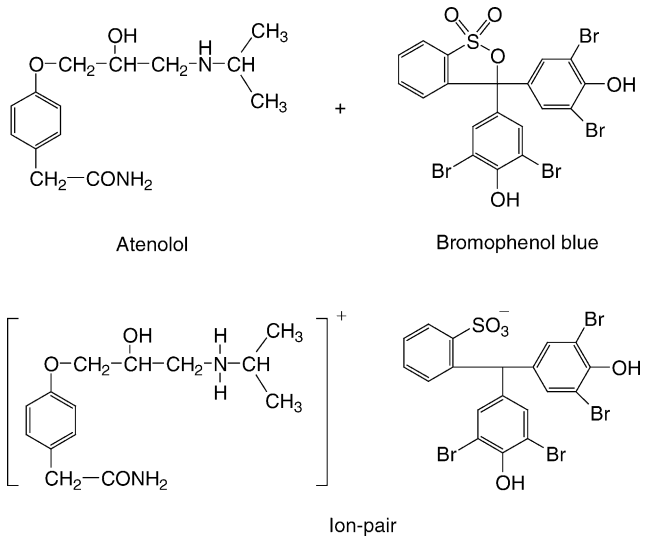



Fig. 2. Effect of volume of dye solution (0.1% on the development of the reaction product of atenolol ($5\,\mu g\,ml^{-1}$) and (A) bromophenol blue, (B) bromothymol blue, (C) bromocresol purple.




Scheme 1. Proposal of the reaction pathway between atenolol and bromophenol blue.

in chloroform are not absorbing light in the visible region, but upon addition of any of the three dyes, they have an absorption maxima at 415 nm (Fig. 1).

The acid dye technique is a general procedure for the quantitative analysis of a variety of pharmaceutical amines [24–26]. In practice, a buffered aqueous solution containing the amine and a suitable indicator dye is shaken with an organic solvent. The concentration of the resulting ion-pair in the organic phase is then determined spectrophotometrically. The ion-pair extraction technique leads sometimes to emulsion formation, difficulties in reproducibility when extraction is carried out in a separator and, is time-consuming. Few studies have reported the analysis of pharmaceutical compounds through formation of ion-pair, without extraction, followed by spectrophotometric [27,28].

Table 1
The stability constants and free energy changes ($\Delta G$) of the studied compounds with acid dyes

| Compound | BPB | | BTB | | BCP | |
|---|---|---|---|---|---|---|
| | $\log K_F$ | $-\Delta G$ (J) | $\log K_F$ | $-\Delta G$ (J) | $\log K_F$ | $-\Delta G$ (J) |
| Atenolol | 3.9291 | 5358.03 | 3.6283 | 4947.83 | 3.1841 | 4342.05 |
| Timolol | 2.7904 | 3805.18 | 3.0311 | 4133.36 | 3.4373 | 4687.32 |
| Nadolol | 3.5570 | 4850.62 | 3.4652 | 4725.41 | 3.5665 | 4863.55 |

Table 2
Tolerance limit of the co-formulated drugs for nadolol (6 μg ml$^{-1}$) using bromophenol blue

| Drug | Tolerance limit (μg ml$^{-1}$) |
|---|---|
| 1. Verpamil | 4.6 |
| 2. Diazepam | 1.14 |
| 3. Theophylline | 3.05 |
| 4. Ibuprofen | 27.5 |
| 5. Benzoin | 3.92 |

Table 3
Analytical parameters for the determination of the studied compounds using the proposed methods

| Parameters | Compound | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Atenolol | | | Timolol | | | Nadolol | | | |
| | BPB[a] | BTB[a] | BCP[a] | BPB[a] | BTB[a] | BCP[a] | BPB[a] | BTB[a] | BCP[a] |
| Linearity range (μg ml$^{-1}$) | 1–8 | 1–9 | 1–10 | 1–10 | 1–10 | 1–10 | 1–10 | 1–10 | 1–10 |
| Molar absorptivity (l mol$^{-1}$ cm$^{-1}$) | $3.3 \times 10^4$ | $2.6 \times 10^4$ | $2.3 \times 10^4$ | $3.8 \times 10^4$ | $3.7 \times 10^4$ | $2.7 \times 10^4$ | $2.5 \times 10^4$ | $3 \times 10^4$ | $2.8 \times 10^4$ |
| Linear regression | | | | | | | | | |
| Intercept ($a$) | −0.0124 | $-8.3 \times 10^{-5}$ | $8.73 \times 10^{-3}$ | $-2.4 \times 10^{-3}$ | $-3.73 \times 10^{-3}$ | $-1.47 \times 10^{-3}$ | $-1.42 \times 10^{-3}$ | 0.0167 | $-2 \times 10^{-4}$ |
| Slope ($b$) | 0.1208 | 0.09528 | 0.08596 | 0.0842 | 0.0841 | 0.0628 | 0.0822 | 0.0961 | 0.0925 |
| Correlation coefficient ($r$) | 0.9991 | 0.9994 | 0.9993 | 0.9995 | 0.9997 | 0.9998 | 0.9981 | 0.9994 | 0.9997 |
| $S_{y/x}$ | 0.01462 | $9.85 \times 10^{-3}$ | 0.0102 | 0.0195 | $5.92 \times 10^{-3}$ | $3.87 \times 10^{-3}$ | 0.0142 | 0.0111 | $7.29 \times 10^{-3}$ |
| $S_a$ | 0.0138 | $9.5 \times 10^{-3}$ | $9.37 \times 10^{-3}$ | 0.0179 | $5.45 \times 10^{-3}$ | $3.57 \times 10^{-3}$ | 0.0131 | 0.0102 | $6.72 \times 10^{-3}$ |
| $S_b$ | $1.95 \times 10^{-3}$ | $1.27 \times 10^{-3}$ | $1.12 \times 10^{-3}$ | $2.14 \times 10^{-3}$ | $6.52 \times 10^{-4}$ | $4.26 \times 10^{-4}$ | $1.56 \times 10^{-3}$ | $1.22 \times 10^{-3}$ | $8.04 \times 10^{-4}$ |
| Detection limit (μg ml$^{-1}$) | 0.3311 | 0.2023 | 0.1165 | 0.4544 | 0.0850 | 0.1370 | 0.3350 | 0.0386 | 0.1670 |

$S_{y/x}$ = standard deviation of residuals; $S_a$ = standard deviation of intercept of regression line; $S_b$ = standard deviation of slope of regression line.
[a] Dye.

### 3.1. Optimization of experimental parameters

BPB, BTB and BCP in chloroform were used for direct determination of atenolol, timolol and nadolol in the same solvent producing a yellowish orange colour peaking at 415 nm (Fig. 1). The experimental factors affecting the development and stability of the product were studied and optimized. Such factors include, concentration of the reagents, time of reaction and standing time. The influence of the concentration of BPB, BTB and BCP was studied using different volumes of 0.1% solutions. The highest results were obtained with 1 ml of each dye Fig. 2. The addition of the dye solutions resulted in an immediate full color development at room temperature and the formed ion pairs were stable for at least 2 h.

### 3.2. Composition of the ion-pair complexes

The composition of the ion-pair associates was established using BPB as a model example for the dye with atenolol by applying Job's method of continuous variations. The plot reached a maximum value at a mole fraction of 0.5 which indicated the formation of 1:1 (drug:dye) complex. The reaction pathway is proposed to proceed as shown in Scheme 1.

The conditional stability constants ($K_F$) of the ion-pair complexes were calculated from the data of mole ratio and continuous variations methods. $\log K_F$ values and the free energy changes ($\Delta G$) for the β-blockers ion-pairs with three dyes are tabulated in Table 1.

The interference likely to be produced from co-formulated drugs was studied by adding 4 μg ml$^{-1}$ of each compound to 6 μg ml$^{-1}$ of nadolol as a model example and using BPB dye. The apparent concentration of the drugs in these samples were determined and the tolerance limit (concentration of interfering drug causing less than 3% relative error) were calculated (Table 2). Although the tolerance limit of these compounds are slightly low, the interference resulting from their presence can be avoided as they are always present as minor components relative to the β-blockers.

The studied compounds are stable at room temperature in the solid state. Prolonged exposure to extremes of temperature and humidity caused degradation of the solid compound. For example, timolol undergoes degradation through one of following ways:

1. rearrangement to isotimolol;
2. ether cleavage to form 4-hydroxy-3-morpholino-1,2,5-thiadizole;
3. oxidation followed by ether cleavage to form 4-hydroxy-3-morpholino-1,2,5-thiadiazole-1-oxide [29]. The last two pathways involve the secondary amino group, hence the degradation product will not interfere with the assay.

### 3.3. Analytical performance

The absorbance–concentration plots were found to be linear over the concentration range stated in Table 3. The

Table 4
Application of the proposed methods and official methods to the determination of studied compounds in pure samples

| Compound | Proposed method | | | | Official method [29,30] | |
|---|---|---|---|---|---|---|
| | Amount taken μg ml$^{-1}$ | BPB % recovery | BTB % recovery | BCP % recovery | Amount taken (mg) | % recovery |
| Atenolol | 2 | 99.14 | 99.58 | 99.42 | 100 | 100.71 |
| | 4 | 99.36 | 99.20 | 100.29 | 150 | 99.72 |
| | 5 | 100.67 | 100.21 | 99.77 | 200 | 99.90 |
| | 6 | 100.56 | 100.35 | 100.95 | 250 | 100.24 |
| | 8 | 100.09 | 100.87 | 100.86 | | |
| Mean ± S.D. | | 99.96 ± 0.62 | 100.04 ± 0.59 | 100.26 ± 0.60 | | 100.14 ± 0.38 |
| *t* | | 0.51 (2.776) | 0.29 (2.776) | 0.35 (2.776) | | |
| *F* | | 2.69 (9.12) | 2.43 (9.12) | 2.51 (9.12) | | |
| Timolol | 1 | 99.94 | 100.92 | 100.56 | 400 | 100.92 |
| | 3 | 99.42 | 99.26 | 100.70 | 600 | 100.76 |
| | 5 | 100.64 | 100.15 | 99.67 | 800 | 101.54 |
| | 7 | 99.57 | 99.56 | 99.94 | 1000 | 100.26 |
| | 9 | 100.53 | 100.76 | 99.58 | | |
| Mean ± S.D. | | 100.02 ± 0.49 | 100.13 ± 0.65 | 100.09 ± 0.46 | | 100.87 ± 0.46 |
| *t* | | 2.65 (2.776) | 1.92 (2.776) | 2.54 (2.776) | | |
| *F* | | 1.16 (9.12) | 2.01 (9.12) | 1.01 (9.12) | | |
| Nadolol | 2 | 100.89 | 99.50 | 100.56 | 250 | 99.57 |
| | 4 | 99.40 | 100.48 | 99.97 | 280 | 100.46 |
| | 6 | 99.59 | 100.17 | 99.95 | 300 | 100.28 |
| | 8 | 100.31 | 99.74 | 99.92 | 320 | 99.68 |
| | 10 | 99.38 | 99.12 | 99.45 | | |
| Mean ± S.D. | | 99.91 ± 0.59 | 99.80 ± 0.48 | 99.97 ± 0.35 | | 99.99 ± 0.38 |
| *t* | | 0.23 (2.776) | 0.64 (2.776) | 0.08 (2.776) | | |
| *F* | | 2.44 (9.12) | 1.60 (9.12) | 1.16 (9.12) | | |

Table 5
Application of the proposed methods and official methods to the determination of studied compounds in dosage forms

| Preparation | Proposed method | | | | Official method [29,30] | |
|---|---|---|---|---|---|---|
| | Amount taken $\mu g\,ml^{-1}$ | BPB | BTB | BCP | Amount taken $mg\,ml^{-1}$ | % recovery |
| Tenormin[a] (atenolol 50 mg, tablet) | 3 | 98.56 | 97.48 | 99.46 | 0.10 | 98.44 |
| | 5 | 99.14 | 98.56 | 99.52 | 0.15 | 98.81 |
| | 7 | 99.32 | 98.27 | 99.57 | 0.20 | 98.50 |
| | 9 | 98.37 | 98.34 | 98.45 | 0.25 | 99.12 |
| Mean ± S.D. | | 98.85 ± 0.39 | 98.16 ± 0.41 | 99.25 ± 0.46 | | |
| *t* | | 0.54 (3.182) | 2.28 (3.182) | 1.97 (3.182) | | |
| *F* | | 2.10 (9.82) | 2.26 (9.82) | 2.92 (9.82) | | |
| Hypoten[b] 50 (Atenolol, 50 mg tablet) | 3 | 97.26 | 98.03 | 98.62 | 0.10 | 97.66 |
| | 5 | 98.20 | 99.58 | 98.45 | 0.15 | 97.62 |
| | 7 | 98.76 | 97.86 | 97.03 | 0.20 | 98.11 |
| | 9 | 98.12 | 98.63 | 98.78 | 0.25 | 98.97 |
| Mean ± S.D. | | 98.09 ± 054 | 98.53 ± 0.67 | 98.22 ± 0.70 | | 97.87 ± 0.23 |
| *t* | | 1.15 (3.182) | 1.86 (3.182) | 0.96 (3.182) | | |
| *F* | | 5.64 (9.82) | 8.87 (9.82) | 9.52 (9.82) | | |
| Cusimolol[c] 0.5% eye drops (timolol 0.5 mg (100 ml)$^{-1}$) | 3 | 101.67 | 100.76 | 99.48 | 0.025 | 101.23 |
| | 5 | 100.94 | 101.23 | 99.34 | 0.05 | 100.67 |
| | 7 | 102.53 | 100.51 | 100.88 | 0.10 | 100.85 |
| Mean ± S.D. | | 101.71 ± 0.65 | 100.83 ± 0.30 | 99.90 ± 0.69 | | 100.92 ± 0.23 |
| *t* | | 1.98 (4.303) | 0.41 (4.303) | 2.41 (4.303) | | |
| F | | 7.75 (19.0) | 1.63 (19.0) | 8.87 (19.0) | | |
| Corgard[d] (nadolol 80 mg per tablet) | 3 | 102.24 | 100.42 | 100.58 | 0.02 | 101.67 |
| | 5 | 101.96 | 100.67 | 101.77 | 0.04 | 100.56 |
| | 7 | 101.53 | 99.89 | 101.35 | 0.06 | 100.67 |
| Mean ± S.D. | | 101.91 ± 0.29 | 100.33 ± 0.33 | 101.23 ± 0.49 | | 100.97 ± 0.50 |
| *t* | | 2.81 (4.303) | 1.86 (4.303) | 0.64 (4.303) | | |
| *F* | | 2.92 (19.0) | 2.36 (19.0) | 1.03 (19.0) | | |

The figures in parenthesis are the tabulated values of *t* and *F* at $p = 0.05$; each result is the average of three separate determinations.
[a] Product of Zeneca Limited, Macclesfield Cheshire, U.K.
[b] Product of Hikma Pharmaceuticals, Amman, Jordon.
[c] Product of Alcon Cusi, S.A. c/Camil Fabra, El Masnou-Barcelona, Spain.
[d] Product of Bristol-Myers Squibb, Egypt.

statistical parameters were given in the regression equation calculated from the calibration graphs, along with the standard deviations of the slope ($S_b$) and the intercept ($S_a$) on the ordinate and the standard deviation residuals ($S_{y/x}$).

The linearity of calibration graphs was proved by the high values of the correlation coefficient (*r*) and the small values of the *y*-intercepts of the regression equations. The apparent molar absorptivities of the resulting colored ion-pair complexes were also calculated and recorded in Table 3.

The proposed methods were applied to the determination of pure samples of atenolol, timolol and nadolol. The results obtained were compared with those given by reference methods [30,31]. Statistical analysis [32] of the results obtained by both methods using the Student's *t*-test and variance ratio, *F*-test, reveals no significant difference in the performance of the two methods regarding accuracy and precision, respectively Table 4.

Formation of the ion pair complex with anionic dye necessitate the presence of a basic function group therefore no possible interference is likely to occur from co-formulated drugs lacking a basic center such as ibuprofen Table 2. Also drugs that are not extractable in chloroform will not interfere in the assay.

Common excipients such as talc powder, lactose, maize starch, avisil hydrogenated vegetable oil, gelatine, magnesium stearate did not interfere with the assay.

The proposed methods have been further applied to analysis of the β-blockers in tablets and eye drops (Table 5). The results were compared statistically, applying the *t*-test and *F*-test with the official methods [30,31]. The results obtained by both methods revealed no significant difference between the performance of the two methods regarding accuracy and precision.

## 4. Conclusion

A simple, fast, extraction-free method is proposed to the analysis of some β-blockers in pure form and in their

dosage forms. Compared with the official methods, the method is very simple, requiring only one reagent, no pH-adjustment and inexpensive instrumentation. The lower quantitation limit (LQL) of the proposed method is much lower (1 $\mu g\,ml^{-1}$) than those of the official method (10, 30 and 50 $\mu g\,ml^{-1}$). The lower detection limit of the proposed method less than that of the reported method [33]. The high sensitivity of these methods allows it to be applied to content uniformity test and single dose analysis.

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