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Short communication

Rapid and selective UV spectrophotometric and RP-HPLC methods for dissolution studies of oxybutynin immediate-release and controlled-release formulations

Manthena V.S. Varma, Aditya M. Kaushal, Sanjay Garg*

National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, SAS Nagar, Punjab 160062, India

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Abstract

A new UV spectrophotometric method and a reversed-phase HPLC method were developed for quantitative evaluation of oxybutynin hydrochloride (OXB) formulations. Determination of OXB by UV spectroscopic method was based on complexation of OXB with picric acid to form picrate, which was extracted to chloroform. The picrate complex showed quantifiable absorbance at 344 nm. Chromatography was carried out at 25 °C on a 4.6 mm × 250 mm 5 μm cyano column that contained USP packing L10 with water:methanol:acetonitrile::48:12:40 (v/v), as mobile phase. UV detector was set at 203 nm. Both methods were found to be selective, linear, accurate and precise in the specified ranges. The LOD and LOQ of HPLC method were 0.5 and 1.65 μg/ml, respectively. Intra-day and inter-day variability for both methods were <2% RSD. These methods were successfully used for quantification of OXB in drug-release studies from immediate-release tablets and controlled-release (CR) formulations.

Keywords: Oxybutynin hydrochloride; UV spectroscopy; RP-HPLC; Dissolution study

1. Introduction

OXB is a tertiary amine ester, [4-(diethylamino)-2-butynyl (±)-α phenylcyclohexane-glycolate hydrochloride] indicated for relief of symptoms associated with uninhibited and reflex neurogenic bladder called urinary incontinence (UI) [1], which is a prevalent and costly condition that affects approximately 38% of older community-dwelling women [2,3]. This compound is a monoprotic base with pK_a value of 8.04 and has a solubility of 0.012 mg/ml for free base at 37 °C [4]. OXB and/or its formulation(s) are official in USP, EP and BP. Being the drug of first choice in treating UI, OXB has been studied extensively for its pharmacodynamic properties [5,6] and pharmacokinetic parameters [7–9].

Two analytical methods, UV spectrophotometry and RP-HPLC, have been developed to quantitatively estimate OXB in pure form and to evaluate OXB matrix-based and controlled-porosity osmotic pump CR formulations. UV spectrometric method was based on ion-pair complexation of picric acid with *tertiary*-amine group of OXB to form an UV active complex. OXB has absorbance maxima at 196 nm [4], where any ingredient or solvent will interfere. Geraghty et al. reported assay of OXB in liquid crystalline gels by spectrophotometric method, analyzed at 230 nm [10]. Ishikawa et al. used 244 nm for analyzing OXB in rapidly disintegrating taste-masked granules [11]. Low absorbance and the interference of excipients at these wavelengths are the two main reasons that can be attributed for the failure of these methods in evaluating OXB formulations in our lab. Several LC methods for the analysis of OXB during preclinical and clinical studies and for evaluating formulations have been developed, however, all these involve long run times with complex extraction procedures [9,12,13]. A simple and specific RP-

* Corresponding author. Present address: School of Pharmacy, The University of Auckland, Private Bag 92-019, Auckland, New Zealand.
Tel.: +64 9 373 7599x82836; fax: +64 9 367 7192.
E-mail address: s.garg@auckland.ac.nz (S. Garg).

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HPLC method, with short run time was developed for the quality control analysis of OXB in compatibility samples, stability samples and also in dissolution samples. Two methods were validated for application to their specific purposes and further drug-release quantification from CR formulations by both the methods was compared.

2. Materials and methods

2.1. Reagents, formulations and equipment

OXB was supplied by Unichem Lab. Ltd. (Mumbai, India) as gratis sample. The purity of the supplied OXB was 99.5% by HPLC analysis. All reagents used were of analytical grade and the solvents used were HPLC grade (J.T. Baker, Mexico). Water used for HPLC was obtained after reverse osmosis (Elga Water Unit, Germany) of triple distilled water.

Ditropan XL 5 mg tablets (Alza Corp., CA, USA) were kindly provided by Unichem Lab. Ltd. (Mumbai, India). Cystran 5 (Intas Pharmaceuticals Ltd., Ahmedabad, India), 5 mg immediate-release OXB tablets were purchased from local retail pharmacy. Other formulations—Oxymat and Oxyos—were in-house formulations.

A LC Shimadzu (Japan) with a LC-10ATVP Shimadzu pump (Japan), UV detector SPD-M10AVP Shimadzu (Japan), and PDA detector SPD-M10AVP Shimadzu (Japan), system controller SCL-10AVP Shimadzu (Japan), Class-VP system manager software. A 4.6 mm × 250 mm 5 μm cyano Hypersil BDS CPS column (Thermohypersil, UK) with 4.6 mm × 10 mm 5 μm Hypersil BDS CPS precolumn (Thermohypersil, UK) was used for elusion.

2.2. Methods

2.2.1. UV spectrophotometric method and its validation

2.2.1.1. Analytical procedure. To each standard/sample solution (15 ml), 5 ml of picric acid solution (PAS) and 10 ml of chloroform were added in 60 ml seperators and shaken for 2 min on a mechanical shaker operated at 50 motions/min. PAS consisted of 100 mg picric acid, 14.5 mg of anhydrous sodium acetate and 20 ml of glacial acetic acid in 480 ml of demineralized water. Chloroform layer was collected separately and scanned for UV spectrum in the range 200–500 nm on UV spectrophotometer (Lambda 20, Perkin Elmer). λ_{max} was traced and calibration curve of absorbance at 344 nm versus concentration of standard solutions was constructed.

2.2.1.2. Method validation. The proposed method was validated for drug specificity, linearity, accuracy and precision. Dummy tablets and excipient blends, which contained selected excipients in the proportions as expected in the final formulations, were used to validate the method for specificity and recovery. Linearity of six standard solutions with concentrations evenly distributed across the range 10–110% of drug assay (i.e. 2, 5, 10, 15, 20, and 22 μg/ml) in the dissolution medium was established. Accuracy and precision was assessed using three replicates of three concentrations in the range and with six replicates for 100% drug release (i.e., 20 μg/ml). To assess intra-day variation, calibration curve was prepared three times on the same day. The procedure was further repeated on three consequent days for determination of inter-day variability [14]. The results were expressed as %RSD of slopes and intercepts and 95% confidence intervals.

2.2.2. RP-HPLC method and its validation

2.2.2.1. Reagents and solutions. Mobile phase consisting of solvent A and acetonitrile in the proportion of 60:40 was prepared, where solvent A was a mixture of water, methanol and triethylamine in the proportion of 3200, 800 and 0.9 ml, respectively, with pH 3.5 (adjusted with phosphoric acid). Mobile phase was filtered through 0.45 μm nylon filter (Millipore) and deaerated in ultrasonic bath (Branson sonicator).

2.2.2.2. Chromatographic conditions. Chromatography was carried out on Shimadzu HPLC equipped with Class VP software for data processing. Samples were analyzed at 25 °C on a 4.6 mm × 250 mm × 5 μm cyano column (Hypersil BDS CPS) attached to a Hypersil BDS CPS precolumn. UV–vis detector (SPD-10A VP) was set at 203 nm and mobile phase was pumped at a flow rate of 1.5 ml/min. Sample injections of 50 μl each were made automatically using autosampler (SIL-10AD VP). For peak purity in the presence of excipients, a PDA detector (SPD-M10A VP) was used.

2.2.2.3. Method validation. RP-HPLC method was validated for drug specificity, linearity, accuracy and precision and system suitability as per ICH guidelines [15]. Further, this method was used to evaluate solution stability. Peak purity and %recovery of OXB spiked in placebo blend were used for assessing specificity of the method.

Calibration curve of OXB in mobile phase prepared using 16, 18, 20, 22 and 24 μg/ml standard solutions were used for assay and to analyze compatibility samples. The limit of detection (LOD) and the limit of quantification (LOQ) were separately determined by diluting the standard concentrations [16–18]. Accuracy and precision was assessed using three replicates of three concentrations in the range and with six replicates for 100% drug release (i.e., 20 μg/ml), in each medium. To assess intra-day variation, calibration curve was prepared twice on the same day. The procedure was further repeated on three consequent days for determination of inter-day variability.

2.2.3. Application of proposed methods

2.2.3.1. Solution stability of OXB. Standard solutions of three different concentrations (10, 15, and 20 μg/ml) were stored in refrigerator (2–8 °C) (n = 4) and at 37 ± 0.5 °C for 24 h and analyzed by HPLC. Percentage agreement of the

solutions after storage was calculated for assessing solution stability.

2.2.3.2. Dissolution studies of OXB formulations. Effectiveness of UV spectroscopic method in estimating OXB was confirmed by comparing with HPLC method. Dissolution studies of an immediate-release marketed product (Cystran 5, Intas Pharmaceuticals Ltd., Ahmedabad, India), a matrix-based CR formulation (Oxymat, in-house), and two osmotic-based CR formulations (Ditropan XL, Alza Corp., USA; Oxyos, in-house), were conducted and the samples obtained were analyzed by both the methods. Assay was carried out for all the formulations using HPLC method. Three tablets from each batch were crushed and contents equivalent to 5 mg was carefully transferred to a 50-ml beaker and repeatedly rinsed with mobile phase to ensure complete transfer (n = 3). The contents of beaker were sonicated for 10 min and transferred to a 100-ml volumetric flask and volume made up with mobile phase, and then diluted to give a concentration of ~20 μg/ml. These samples were filtered through 0.45 μm nylon filter and analyzed by RP-HPLC. Assay content of each formulation was taken as 100% in dissolution studies.

Dissolution studies were conducted using USP type I dissolution apparatus (Electrolab, Mumbai, India) operated at a rotation speed of 100 rpm. Five hundred milliliters of SIF was used as dissolution medium. At each predetermined time points, 20 ml of sample was withdrawn from each vessel and equal volume was replaced with fresh buffer. Samples were analyzed by UV spectrophotometric method and HPLC method (n = 3). Dissolution profiles obtained thereof were compared using f_2 values. The f_2 value is a logarithmic reciprocal square root transformation of one plus the average means squared differences in percentage dissolved between the test (T_i) and reference (R_i) products over time points (n):

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{n} \right) \sum_{j=1}^{n} |R_j - T_j|^2 \right]^{-0.5} \times 100 \right\}$$

Center for Drug Evaluation and Research, USFDA and Human Medicines Evaluation Unit of the European Agency for the Evaluation of Medicinal Products (EMEA) suggests that two dissolution profiles are similar if f_2 is between 50 and 100 [19].

3. Results and discussion

3.1. UV spectrophotometric method

UV scans of picrate, at different concentrations, in chloroform over a range 200–500 nm are given in Fig. 1a. Volume of drug solutions and chloroform were kept as 15 and 10 ml, respectively, so as to obtain high absorbance values. A study

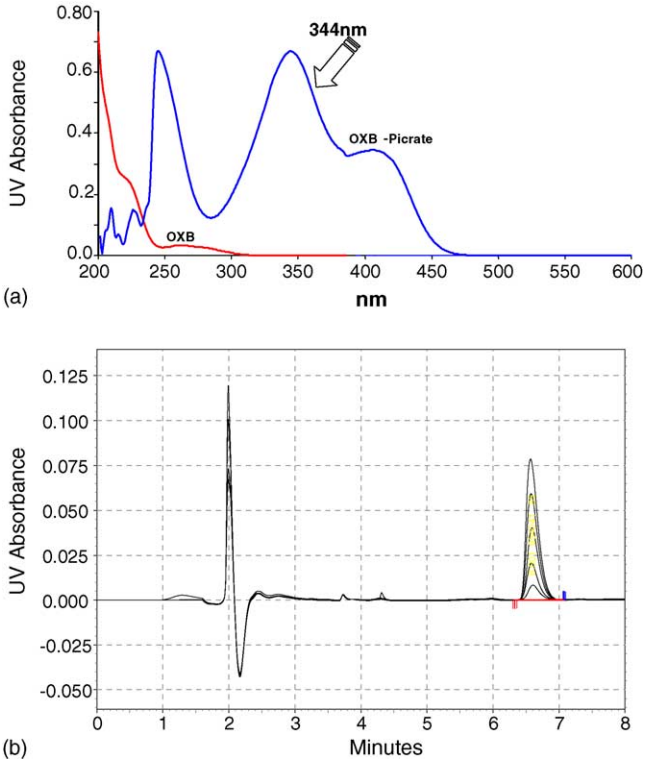

Fig. 1. (a) UV scans of OXB solution (10 μg/ml) in phosphate buffer and its picrate in chloroform. (b) Chromatograms of OXB in pH 6.8 phosphate buffer.

was conducted to check the partitioning of picrate into chloroform. In this study, after extraction of 20 μg/ml solution, chloroform was completely removed and the aqueous phase was re-extracted using 10 ml of fresh chloroform. Absorbance of 0.0030 (<1%) was observed, which indicate complete partitioning of picrate into chloroform and that 10 ml chloroform is sufficient to extract out the drug even at maximum concentration.

3.1.1. Method validation

3.1.1.1. Specificity. Specificity of the method was validated from the recovery of OXB spiked in the placebo blend. %Recovery (101.4) and %RSD (1.92) values of 20 μg/ml drug solution show validity of method for recovery. %Agreement (0.61 ± 0.46%) of solutions containing dummy tablets powder, which was not spiked with drug, showed that the interference of excipients is negligible.

3.1.1.2. Linearity and range. Linearity of the method was observed in expected concentration range 2–22 μg/ml (10–110%) in pH 6.8 phosphate buffer. Statistical analysis of the calibration curve was done and the results are summarized in Table 1. Correlation coefficient (r^2 = 0.9999) shows the validity of Beer's law. Percentage intercept was less than 2% of the response of 100% of test concentration in all the cases, indicating functional linearity between the concentration of analyte and the absorbance.

Table 1
Statistical analysis for calibration curves of OXB by UV and HPLC methods

	UV	HPLC		
	Phosphate buffer of pH 6.8)	Mobile phase	Phosphate buffer of pH 6.8	SGF
Analytical wavelength (nm)	344	203	203	203
Linearity range (μg/ml)	2–22	16–24	2–22	2–22
Regression equation	$Y = 0.0342X - 0.0008$	$Y = 51753X + 22308$	$Y = 53097X + 8906.8$	$Y = 49210X - 8055$
S.D. of slope ($n = 3$)	0.0005	921	446	482
S.D. of intercept ($n = 3$)	0.0002	7216	3805	8860
% intercept	0.10	2.11	0.83	0.81
Correlation coefficient	0.9998	0.9996	0.9998	0.9989
95% confidence intervals				
Slope	0.03352 to 0.03493	49820 to 53690	51740 to 54400	46260 to 52160
Intercept	−0.01097 to 0.00928	−1478 to 46090	−7095 to 24910	−44260 to 28150

Table 2
Accuracy and precision of UV spectroscopy and HPLC methods

Method	Medium	Drug concentration (μg/ml)	Response	
			%Agreement	%RSD
UV Spectroscopy	Buffer (pH 6.8)	5 ($n = 3$)	100.85	1.46
		10 ($n = 3$)	101.98	1.88
		15 ($n = 3$)	99.11	2.88
		20 ($n = 6$)	99.14	0.60
HPLC	Mobile phase	16 ($n = 3$)	99.38	0.54
		24 ($n = 3$)	101.33	0.62
		20 ($n = 6$)	99.47	1.40
	Buffer (pH 6.8)	5 ($n = 3$)	98.42	0.51
		10 ($n = 3$)	98.38	1.59
		15 ($n = 3$)	102.45	0.12
		20 ($n = 6$)	102.54	1.052
	SGF	5 ($n = 3$)	96.84	1.60
		10 ($n = 3$)	99.86	0.68
		15 ($n = 3$)	100.21	1.90
		20 ($n = 6$)	99.60	1.91

3.1.1.3. Accuracy and precision. Accuracy and precision are demonstrated by %recovery and %RSD of three replicates of 5, 10, and 15 μg/ml solutions and six replicates of 20 μg/ml solutions (Table 2).

3.1.1.4. Intra-day and inter-day variability. Results of intra-day and inter-day precisions are given in Table 3. UV spectroscopic method passed the intermediate precision of intra-day and inter-day variability as %RSD of slopes of calibration curves, when analyzed on same day ($n = 3$) and on three consecutive days, was within 2%.

3.2. HPLC method

A reversed-phase HPLC method was developed with a specific procedure for the quality control analysis of OXB in

Table 3
Intra- and inter-day precision of OXB standards

	Intra-day precision				Inter-day precision		
	UV	HPLC			UV	HPLC	
	Buffer (pH 6.8)	SGF	Buffer (pH 6.8)	Mobile phase	Buffer (pH 6.8)	Buffer (pH 6.8)	Mobile phase
Slope							
Average	0.0342	53097	49210	51753	0.0335	48275	52610
%RSD	1.69	0.84	0.980	1.782	1.951	1.121	1.459
Intercept							
Average	0.0008	8906.8	8055.7	22308	0.0072	5474	20308
%RSD	35.82	42.74	110.35	32.35	86.51	39.33	1.69

compatibility samples, stability samples and in dissolution samples of matrix-based CR formulations. As shown in Fig. 1b, OXB has a retention time of 6.8 min and the area of the peak is proportional to concentration of OXB. Optimum wavelength for detection of OXB in different media was 203 nm at which much better detector responses were obtained.

3.2.1. Method validation

3.2.1.1. Specificity. Specificity of the proposed method was performed by analyzing powdered dummy tablets spiked with appropriate amounts of drug (10 mg) diluted accordingly to obtain 5, 10, and 20 μg/ml drug solutions. %Recovery of all three concentrations was within 96.7 and 100.3%. Further, well-resolved peak of OXB at 6.8 min without any interfering peaks indicates the specificity of the method (Fig. 1b). Peak purity of OXB was studied using PDA in the presence of excipients [20]. Short run time, peak symmetry and peak purity are the advantages that were found with the present HPLC method over the existing methods (data not shown). Short run time is of particular interest during dissolution studies of CR formulation since a large number of samples are generated in each study.

3.2.1.2. Range and linearity. The calibration range in each medium was established by considering the practical range necessary for dissolution or assay, and to give accurate and precise results with good linearity. Detector response (area of peak) was plotted against concentration to obtain calibration curves. For assay of OXB in CR tablets, concentration range 80–120% drug concentration (16, 18, 20, 22, and 24 μg/ml) in mobile phase was used. Whilst for analyzing dissolution samples, concentration range 10–110% (2, 5, 10, 15, 20 and 22 μg/ml) drug solution in pH 6.8 phosphate buffer and SGF was used. Regression analysis was carried out on calibration curves and results are summarized in Table 1. Linearity of the calibration curves and the adherence to Beer's law were validated by the high value of the correlation coefficient.

3.2.1.3. LOD and LOQ. LOD and LOQ were determined at a signal-to-noise ratio of 3 and 10, respectively, using Class-VP software (Shimadzu, Japan). LOD and LOQ were experimentally verified by diluting known concentrations of OXB in phosphate buffer till the peak obtained has more than 3- and 10-fold area of the S.D. of six determinations [16]. LOD and LOQ were found to be 0.5 and 1.65 μg/ml, respectively.

3.2.1.4. Accuracy and precision. Accuracy and precision of the proposed method were assessed as defined by ICH guidelines. Quantitative determinations of three standard solutions in three replicates within the specified range and six replicates at 100% (20 μg/ml) drug concentration were performed and the results are in good agreement. %Agreement and %RSD show the validity for accuracy and precision (Table 2).

3.2.1.5. Intra- and inter-day variations. Table 3 gives the results obtained for intra-day and inter-day precisions. The percentage standard deviation (%RSD) of slopes for each standard curve obtained within day and between three consecutive days proves intermediate precision of inter-day and intra-day validations.

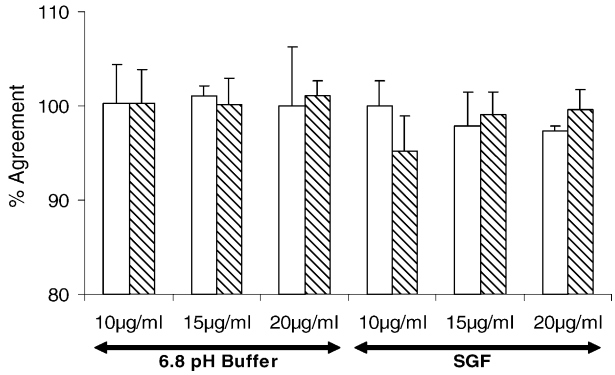

Fig. 2. Solution stability of OXB analyzed by HPLC method. Plain bars indicate the %agreement of standard solutions stored in refrigerator (2–8 °C) for 24 h and cross-hatched bars indicate the %agreement of standard solutions stored at 37 ± 0.5 °C for 24 h. Values are mean ± S.D. of four replicates.

3.3. Applications of the proposed methods

3.3.1. Solution stability

Solution stability of OXB in pH 6.8 phosphate buffer and in SGF was established using HPLC method (Fig. 2). Calculated concentration and percentage agreement of drug solutions (10, 15, and 20 μg/ml) stored at refrigerating and room temperatures were obtained using freshly prepared calibration curves. Results show good agreement and indicate stability of OXB solutions for more than 24 h. These results are insistence to the previous reports [4]. OXB degradation shows pH dependence where OXB is very stable and the degradation is negligible for at least 48 h at acidic to neutral pH conditions. Degradation in alkaline pH was found to be first-order with respect to hydroxide ion concentration. The half-life of degradation at pH 12 was reported as 14 min.

3.3.2. Dissolution studies of OXB formulations

The proposed methods were applied to quantitatively estimate OXB release from an immediate-release (Cystran 5), a matrix-based CR (Oxymat), and two osmotic-based CR formulations (Ditropan XL and Oxyos). UV spectroscopic method showed satisfactory comparable results to that of the RP-HPLC method (Fig. 3). The results of analysis of the commercial tablets and the in-house formulations suggested that there is no interference from any excipients that are used in these formulations. Statistical analysis of the drug-release profiles obtained by both the methods was performed using similarity factor. Similarity factor, f_2 value, is 64.59, 76.19, 73.14, and 76.64 for Cystran 5, Oxymat, Ditropan XL and Oxyos, respectively, indicating that the profiles generated by both the methods are similar.

In conclusion, the proposed UV spectroscopic and RP-HPLC methods provides simple, accurate and reproducible

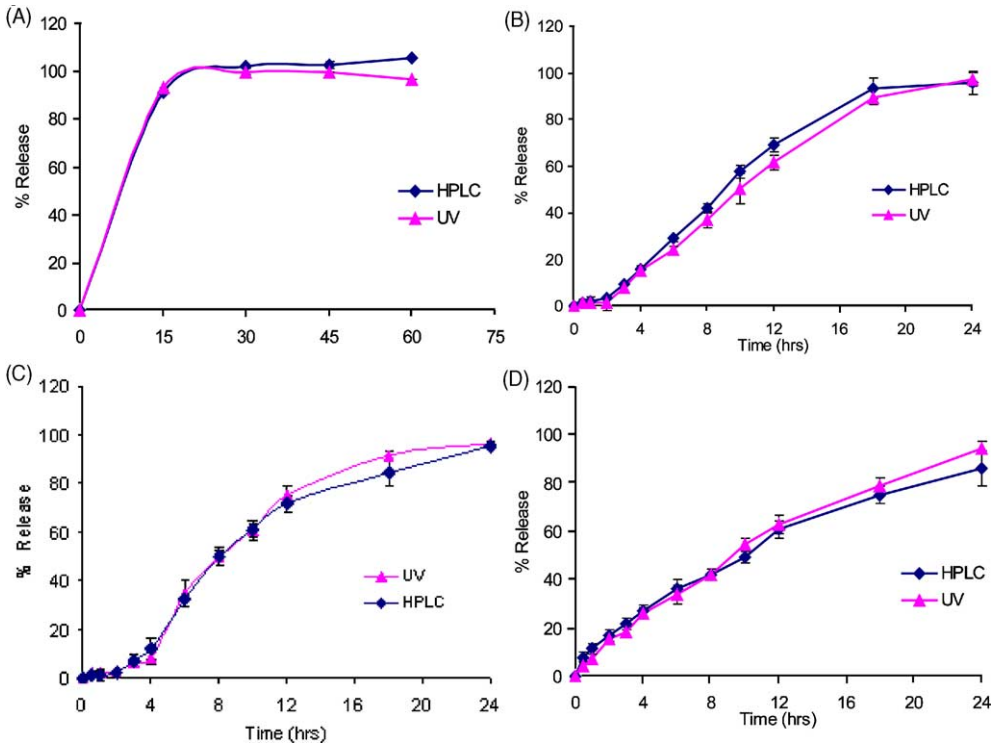

Fig. 3. Comparison of dissolution profiles of (A) immediate-release tablets (Cystran 5), (B) osmotic-based CR formulation (Ditropan XL), (C) osmotic-based CR formulation (Oxyos) and (D) matrix-based CR tablets (Oxymat) generated using UV and HPLC methods.

quantitative analysis for determination of oxybutynin hydrochloride in drug release studies. Proposed methods complied with ICH validation criteria and showed comparable results. UV spectroscopic method has the advantages of lower cost and feasibility, and can be used for routine dissolution studies during CR formulation development.

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