Analytical Validation and Stability Indicating Studies for Simultaneous Estimation of Serdexmethylphenidate and Dexmethylphenidate by RP-HPLC in Bulk and Capsules

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A stability-indicating RP-HPLC method for the simultaneous determination of Serdexmethylphenidate (SER) and Dexmethylphenidate (DEX) has been developed and validated in bulk and tablet dosage forms. SER and DEX are central nervous system stimulants. This combination is used to treat attention deficit hyperactive disorder. The chromatographic analysis was carried out on a Waters C18 Column with 250mm x 4.6 and a particle size of 5 µm, using an isocratic mobile phase of Phosphate Buffer pH 4.8: Methanol (70:30, v/v) at a flow rate of 0.8 mL/min, and the eluents were monitored at an isosbestic point of 215 nm. Specificity, precision, accuracy, linearity, and robustness of the proposed method were all validated according to ICH standards. Forced degradation studies confirmed the method's stability indicating nature. SER and DEX had retention times of 2.390 and 4.602 min, respectively. The developed technique was found to be specific and accurate. SER linearity was achieved between 90-270 µg/mL, while DEX linearity was obtained between 17.50-52.50 µg/mL. SER had LOD and LOQ of 6.35 and 21.17 µg/mL, whereas DEX had LOD and LOQ of 1.18 and 3.93 µg/mL, respectively. As a result, the suggested HPLC method for the quantification of Serdexmethylphenidate (SER) and Dexmethylphenidate (DEX) was reliable, repeatable, accurate, and sensitive.

Keywords: serdexmethylphenidate,dexmethylphenidate, phosphate Buffer, methanol and validation

Attention Deficit/Hyperactivity Disorder (ADHD) is a neuro-developmental disorder marked by persistent symptoms of inattention, hyperactivity, and impulsivity that often begin around the age of seven [1, 2]. Learning, social and familial connections and self-esteem are among the cognitive and behavioural impairments associated with ADHD [3]. Some patients, on the other hand, are mostly inattentive, while others are hyperactive or impulsive [4]. Once thought to be a one-of-a-kind childhood disease, it is now known that 30 to 50 percent of children with ADHD have symptoms that last into adulthood. The continuation of ADHD into adulthood is frequently linked to problems or outcomes in one or more domains. ADHD can raise the likelihood of school or career failure, criminal conduct, drug misuse, and the development of antisocial personality disorder, whether or not it is managed poorly [5, 6].

For several decades, psychostimulants have been the therapy of choice for ADHD [7] Serdex-methylphenidate (SER) is a d-methylphenidate (d-MPH) prodrug [8] that has been authorised for the treatment of ADHD when used in conjunction with Dexmethylphenidate hydrochloride (DEX) [9] When taken orally as directed, SER provides a long-lasting effect due to the slow conversion of the prodrug to active d-MPH. Some stimulant abusers modify oral dose forms to make non-oral delivery easier [for example, intranasal, IV]. Since 1955, racemic immediate-release (IR) methylphenidate hydrochloride (d, 1-MPH) has been used to treat ADHD in children. The pharmacologically active pure chiral d-threo enantiomer of racemic d, 1-MPH is dexmethylphenidate hydrochloride (DEX) [7-8]

Dexmethylphenidate, a CNS stimulant, and Serdexmethylphenidate, a prodrug of dexmethylphenidate, are included in AZSTARYS (serdexmethylphenidate and dexmethylphenidate) capsules. AZSTARYS capsules for oral administration contains 28/6, 42/9, or 56/12 mg serdexmethylphenidate hydrochloride / demethylphenidate hydrochloride (equal to 28/6, 42/9, or 56/12 mg serdexmethylphenidate hydrochloride / demethylphenidate hydrochloride).

According to a study of the literature, only one analytical method was reported by Subrahmanyam Talari *et al.,*[8] the reported retention time of DEX was 4.258 min and for SER 5.629 mins with a total run time of 10 mins. The linearity established for DEX and SER were 0.6-9.0 µg/mL and 2.8-42.0 µg/mL

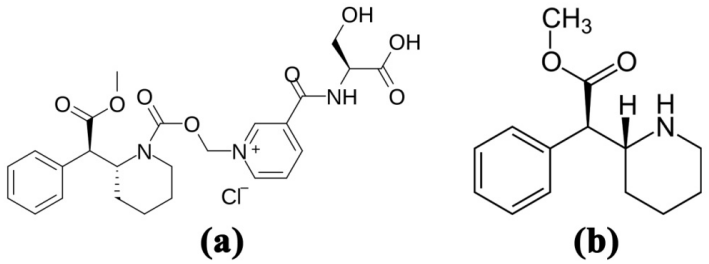

Figure 1. Chemical structures of (a) Dexmethylphenidate and (b) Serdexmethylphenidate.

respectively, which was very narrow. Furthermore, the time consuming and laborious extraction steps utilizing the various buffers (ammonium acetate buffer and orthophsphoric acid buffer) described in the method also makes it a tedious process to implement. This method does not report forced degradation studies too. No other methods were reported in the literature as on date. Given the rising global demand for the aforementioned drugs and the drawbacks of the previously reported methods makes it necessary to develop a new economical, accurate, and rapid HPLC analytical method which will take less time for analysis of simultaneous estimation of both drugs in the pharmaceutical formulation, as well as to conduct forced degradation studies in five different conditions that could be used to evaluate the quality, efficacy, and storage conditions of each drug.

Experimental part

Chemicals and reagents

Both drug standards were gifted from spectrum labs, Hyderabad, India. Methanol, water and acetonitrile (LC grade) were purchased from Sigma-Aldrich. Analytical grade sodium hydroxide (NaOH), hydrogen peroxide (H2O2), hydrochloric acid (HCl) and a 0.22 mm membrane filter were purchased from Sigma-Aldrich. AZSTARYS capsules containing SER and DEX with the label claim of 52.3 mg and 10.4 mg respectively were purchased from the native pharmaceutical market. All chemicals were analytical or LC grade.

HPLC instrumental condition

The technique was developed using an Acquity HPLC system (Waters, Milford, MA, USA) with a model 2996 PDA detector and Empower-2 software. The two analytes were separated by using Phosphate Buffer pH 4.8: Methanol (70:30, v/v) in isocratic mode at a flow rate of 0.8 mL/min at room temperature. The PDA detector was used to monitor the two drugs at 215 nm. Before being used, the solvents were filtered through a 0.22 mm membrane filter and degassed in an ultrasonic bath. Mobile phase was used as diluent.

Preparation of standard solutions

A standard stock solution of SER (180 µg/mL) and DEX (35 µg/mL) was produced by precisely weighing 10 mg of each and dissolving in a 10 mL volumetric flask containing 5 mL of methanol, then sonicating the flask to dissolve the contents and making up to the mark with the same. These samples were aliquoted into a 10 mL volumetric flask with 5 mL of diluent (mobile pahse), sonicated for 5 min, and the remaining volume was brought up to the mark with diluent to get final concentrations of 180 µg/mL for SER and 35 µg/mL for DEX, respectively.

Analysis of formulation

In a mortar, contents of twenty capsules were precisely weighed and crushed into fine powder. A quantity equivalent to 10 mg of DEX was transferred in a volumetric flask, 5 mL of diluent was added and sonicated to ensure the solubility. Finally, the volume was increased to 10 mL in order to obtain the capsules primary stock solution. A 0.35 mL aliquot was taken and transferred to a 10 mL volumetric flask and diluted with mobile phase to obtain concentration of 180 µg/mL for OLA and 35 µg/mL for DEX, respectively. If necessary, the resultant solution was filtered using 0.45 m Millipore nylon filter paper. 10 µL was injected into the HPLC apparatus, and peak areas for SER and DEX were measured. The formulation's % assay was determined.

Validation of chromatographic method

The developed method was validated as per the guidelines of ICH (ICH Guidelines, Q2 (R1), 2005) [9-10].

System suitability

System suitability parameters were measured to verify system performance. The precision of the system was determined in six repeated injections of standard preparations. All important characteristics were measured, including the area of the peak, retention times, tailing factor, the resolution of the peaks and the theoretical plate number.

Accuracy (recovery)

Accuracy is represented (ICH Guidelines, Q2 (R1), 2005) and determined by recovery experiments. In this process, it was tested at three different levels that were 50, 100 and 150% and analyzed chromatogram.

Specificity

To assess the specificity, working placebo solution (blank) in the absence of the SER and DEX and standard solution having a concentration of 180 µg/mL and 35 µg/mL for SER and DEX respectively, as well as formulations were injected into the HPLC system and analyzed chromatograms.

Precision

Precision (Intraday and Interday) of the analytical technique was proven by using optimized concentration

of SER and DEX by six replicate injections. % RSD of Assay, peak areas were determined from chromatograms.

Linearity

Linearity was confirmed by preparing and analyzing the pure analytical standard preparation at five totally different concentrations. The developed method displays ideal linearity over a range of 90.00, 135.00, 180.00, 225.00, 270.00 µg/mL and 17.50, 26.25, 35.00, 43.75, 52.50 µg/mL for SER and DEX respectively.

Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ of SER and DEX were determined by using signal to noise approach as defined in International Conference on Harmonization (ICH) guideline (ICH Guidelines, Q2 (R1), 2005).

Robustness

The impact of minor changes in flow rate (5%), column temperature (5%), and detection wave length (2 nm) on resilience as a measure of method ability to stay unaffected by small, but purposeful changes in chromatographic circumstances was investigated.

Forced Degradation studies

The ICH (ICH Guidelines, Q1A (R2, 2003; D.W. Reynolds et al., 2002) guideline [16] entitled stability testing of new drug substances and products requires that stress testing is performed to describe the inherent stability characteristics of the active substance. The goal of this project was to carry out the stress degradation studies on the SER and DEX by using the proposed method.

Acidic and alkaline hydrolysis

From primary stock solution, transfer 0.35 mL to two pair of 10 mL standard flask. To the above solution 1 mL of 0.1 N HCl was added to one pair of 10 mL standard flask for acidic condition. For alkaline degradation, 1 mL of 0.1 N NaOH was added in another set of 10 mL standard flasks. Then, the standard flask was kept in a water bath at 90 °C for 3 h and 80 °C for 5 h for acid and alkaline samples respectively. Both set of solutions were neutralized and made up to 10 mL with diluent to get 180 µg/ml and 35 µg/ml for SER and DEX respectively. Cool the resulting solution to room temperature. Filter the solution with 0.22 mm syringe and then introduce in the vials of the HPLC system.

Oxidative degradation

Transfer 0.35 mL of primary stock solution to a 10 mL standard. Add 5 mL of 3% (w/v) of hydrogen peroxide and the volume was made up to the mark with diluents to get 180 µg/mL and 35 µg/mL for SER and DEX respectively. The standard flask was heated at 80°C for 30 min. The resulting solution was cooled and introduced in the vials of the HPLC system, after the filtration with 0.22 mm syringe filter.

Thermally induced degradation

Transfer 0.35 mL of primary stock solution to a 10 mL standard flask and the volume was made upto the mark with diluent to get 180 µg/mL and 35 µg/mL for SER and DEX respectively. The resulting solution was refluxed at 85°C for 6 h. Then the solution was cooled to room temperature. Introduce in the vials of the HPLC system, after the filtration with 0.22 mm syringe filter.

Photo degradation

From the stock solution, pipette out 0.35 mL to a 10 mL standard flask and the volume was made up to the mark with diluent to get 180 µg/mL and 35 µg/mL for SER and DEX respectively. Then, the samples were transferred to a Petri dish and set aside in a photostability chamber 200 Wh/m2 in UV light and 1.2 million Lux hours in UV light for 24 h. Cool the final solution to room temperature. Filter the solution with 0.22 mm syringe and then introduce in the vials of the HPLC system.

Results and discussion

Optimization of chromatographic conditions

Numerous chromatographic trails have been carried out depending on the molecules' physico-chemical characteristics. During the trials, four trustworthy factors were taken into account: the stationary phase, mobile phase composition, flow rate, and column temperature. One variable was kept constant while another value was changed to begin the trail. Methanol – ammonium acetate buffer, 50:50 (V/V), acetonitrile - methanol, 30:70 (V/V), methanol - orthophosphoric acid buffer (pH 4.5–6.5), 50:50 (V/V), Methanol: phosphate buffer (pH 3.0–6.5), 25:75 (V/V), and acetonitrile - phosphoric acid buffer (pH 3.2-4.5) 60:40 (V/V) were among the sensitivity of the test, appropriateness for stability studies, simplicity of preparation, and use of widely accessible solvents were all factors in determining the mobile phase's acceptability. As a result, the mobile phase of Phosphate Buffer pH 4.8: Methanol (70:30, v/v) has been shown to be the most effective for isocratic simultaneous estimation of SER and DEX. The detection wavelength was determined by scanning the typical SER and DEX solution between 200 and 400 nm and the isosbestic point of 215 nm was finalized to determine the analytes.

SER and DEX samples have been confirmed as a function of retention time compared to the SER and DEX standard. Furthermore, SER and DEX were detected by adding the standard to the sample before analysis, which resulted in a proportionate increase in the peak area of the sample. At a flow rate of 0.8 mL min^{-1}, the mean SER and DEX retention times were 2.390 and 4.602 min, respectively. Other degradation products did not cause any interference. The blank, standard, and sample chromatograms of SER and DEX are shown in Figure 2 (a-c).

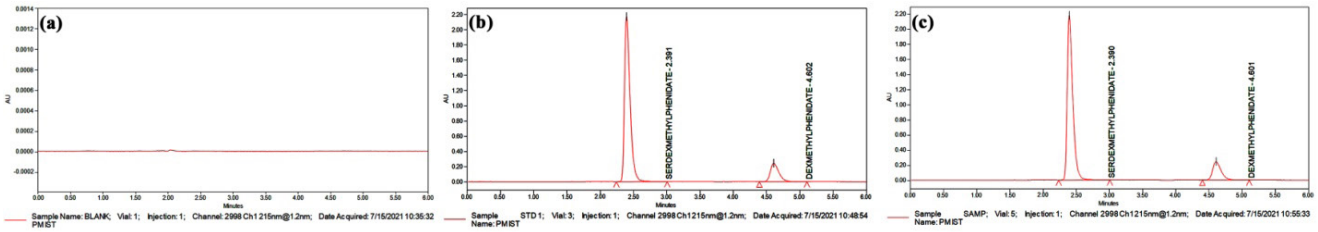

Figure 2. Representative Chromatograms of (a) Blank; (b) Standard; (c) Sample (capsules).

Validation of HPLC Method

System suitability study

System suitability was attained by checking various parameters and found within the ICH limit. The average theoretical plant count for SER and DEX were more than 4000. SER was eluted at 2.390 min and DEX at 4.602 min. The tailing factor for both the analyte peaks were less than 2%.

Accuracy (recovery)

Accuracy was ensured at three different levels that were 50, 100 and 150 %. The results are shown in Table 1. Mean % Recoveries for SER at 50, 100 and 150 % were found to be 99.24, 99.66 and 99.84 % respectively. Similarly, for DEX 100.13, 99.75 and 100.77 % at 50, 100 and 150 % respectively.

Precision

Precision of the analytical method were established for both intra and interday by using concentration of 180 µg/mL and 35 µg/mL by of SER and DEX six replicate injections. The results are shown in Table 2.

Specificity

The specificity of the technique was established that the chromatogram of the working placebo solution did not show any interference at the retention time of the SER and DEX. Thus, it can be concluded that main excipients that were present in the formulations as excipients does not interfere with the analytical method for the determinations of SER and DEX. The resulted chromatogram of blank, standard and formulation are shown in Figure 2 (a-c).

Linearity

The developed method displays ideal linearity over a range of 90.00, 135.00, 180.00, 225.00, 270.00 µg/mL and 17.50, 26.25, 35.00, 43.75, 52.50 µg/mL for SER and DEX respectively. The excellent coefficient correlation was more than 0.999 for both drugs. Residual plot of both drugs displays that residuals are randomly placed over, below and above the x-axis signifying that developed method is a linear model.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection of SER and DEX was found to be 6.35 and 1.18 µg/mL and limit of quantification 21.17 and 3.93 µg/mL respectively indicating that the method was extremely rapid and sensitive. Figure 3 (a ,b) shows the chromatograms of LOD and LOQ.

Table 1. Recovery study.

SERDEXMETHYLPHENIDATE					
S. No	**Accuracy Level**	**Wt. of Sample**	**Amount Added**	**Amount Found**	**% Recovery**
1	50%	192.48	88.41	87.77	99.29
2	100%	384.95	176.81	176.44	99.79
3	150%	577.43	265.22	264.65	99.79
DEXMETHYLPHENIDATE					
4	50%	192.48	17.44	17.45	100.08
5	100%	384.95	34.87	34.87	99.98
6	150%	577.43	52.31	51.94	99.30

* average of 6 replicates; # average of 3 replicates

Table 2. Precision study.

Precision	**% RSD of peak area***		**Mean Assay***		**% RSD of Assay***	
	SER	**DEX**	**SER**	**DEX**	**SER**	**DEX**
Intraday	0.52	0.69	100.55	99.28	0.52	0.69
Interday	0.55	0.66	100.46	99.64	0.55	0.66

* mean of six determinations

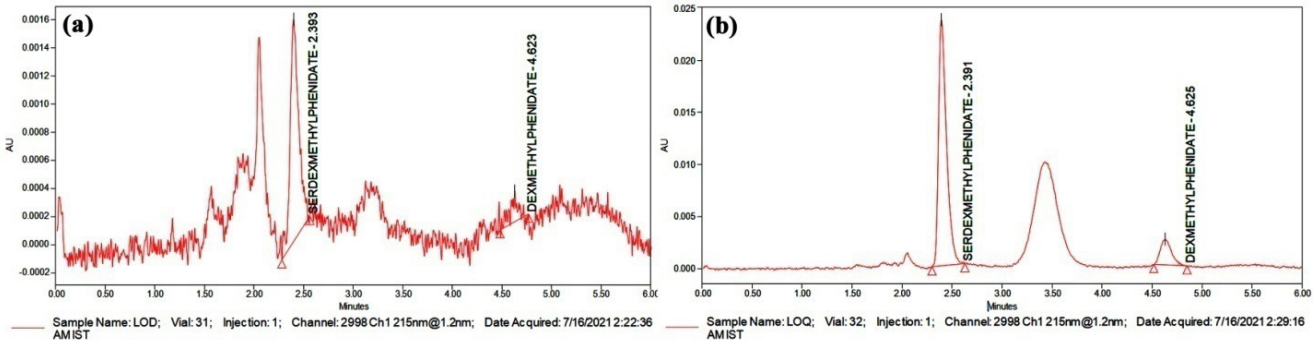

Figure 3. Chromatograms of (a) LOD and (b) LOQ.

Robustness study

The study reveals that there was no much deviation in the robust chromatograms in assessment with optimized one. The results are shown in Table 3.

Assay of formulations

Marketed formulation was analyzed, and assay percentage was calculated. Results were obtained within ICH limit and summarized in Table 2.

Forced degradation study

Forced degradation studies are a useful technique for predicting drug stability issues and identifying the main degradation products during analytical method development. In the presence of degradation products transported in bulk and medicinal dosage form, degradation tests demonstrate the specificity of the proposed method. Purity angles were used to determine the purity of drug peaks in a combination of two medicines. All of the forced degradation conditions listed in the ICH recommendation was applied to the Serdexmethylphenidate and Dexmethylphenidate formulation: hydrolysis (acidic with 0.1 M HCl and basic with 0.1 M NaOH), oxidation (3% H_2O_2), photolysis, and heat stress conditions.

For acid and alkaline samples, 10% degradation was detected after a given length of time at 90 °C for 3 h and 80 °C for 5 h, respectively. A prominent degradation peaks were observed in both the conditions. The reduction in the peak area also represents the detoriation of drugs. Peroxide, thermal, and light deterioration all showed significant decline. When treated with peroxide, both analytes were shown to be extremely unstable with a significant degradation product eluting near 3.5 min with 3 % H_2O_2 at 80 °C for 30 min. The deterioration peak was seen on the chromatograms in Figure 4 (a-e). Under milder oxidative conditions (3 % H_2O_2 at 60 °C for 10 min), the analytes were reasonably stable (95.02 percent). Heat deterioration was a problem for the analytes. Dry heat degradation (85 °C for 6 h) decreased unaltered drug content to approximately 90.47 and 91.08 % for SER and DEX, respectively, with an unknown degradation product. The degradation product was seen in the chromatogram (Figure-4 d). When subjected to 1.2 million Lux hours of near-UV radiation of 200 Wh/m2 for 24 h, SER and DEX were shown to be extremely unstable. Figure 4 (e) depicts the chromatogram. The chromatogram showed elution of a significant degradation product near 3.5 min. According to the findings of the degradation tests, long-term storage of the medication under photolytic conditions causes deterioration, with a decrease in analyte content and a corresponding increase in degradation products. Table 4 summarizes the findings from the SER and DEX stability experiments.

Table 3. Robustness data.

Parameter	Condition	Serdexmethylphenidate		Dexmethylphenidate	
		RT	% Assay	RT	% Assay
Flow	0.6 ml/min	3.181	100.72	6.098	98.58
	0.8 ml/min	2.390	99.11	4.602	100.00
	1.0 ml/min	1.922	99.35	3.753	99.15
Temp	25 °C	2.394	101.40	4.663	100.50
	30 °C	2.390	99.11	4.602	100.00
	35 °C	2.403	98.87	4.642	98.68
Wavelength	213 nm	2.390	98.54	4.601	100.68
	215 nm	2.390	99.11	4.602	100.00
	217 nm	2.391	100.17	4.602	100.35

Table 4. Data of Stress studies.

Condition	Serdexmethylphenidate		Dexmethylphenidate	
	% Assay	% Degradation	% Assay	% Degradation
Acid	89.49	10.51	90.60	9.40
Base	90.04	9.96	91.59	8.41
Peroxide	90.37	9.63	91.93	8.07
Thermal	90.47	9.53	91.08	8.92
UV	90.18	9.82	91.15	8.85

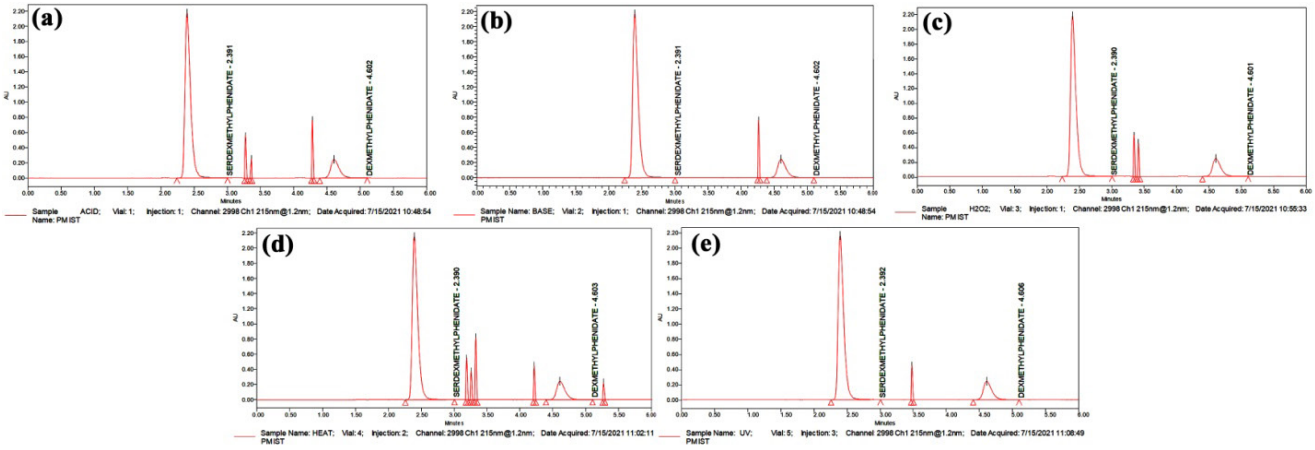

Figure 4. Chromatograms of (a) Acid; (b) Base; (c) Peroxide; (d) Thermal and (d) Photolytic (UV light) degradation studies.

Conclusion

The existing method for the estimation of SER and DEX utilizes Agilent C_{18} column with dimensions of 150 x 4.6mm, 3.5 was used for chromatography and pH-2.5 ammonium acetate buffer with orthophosphoric acid: acetonitrile in a 50:50 v/v pumped at 1 mL/min. The current method utilizes Waters C_{18} Column with 250mm X 4.6 and a particle size of 5 µm, using an isocratic mobile phase of Phosphate Buffer pH 4.8: Methanol (70:30, v/v) at a flow rate of 0.8 mL/min. The process of preparing mobile phase was easy and economical too. Due to the use of orthophosphoric acid buffer the base line was also noisy which was eliminated in the current method by the saturation of column with phosphate buffer pH 4.8, which was close enough to the pK_a of drugs. The pH 2.5 in the previous method makes the column more acidic and there precipitating the drugs in the column. The previous method also uses a separate diluent, whereas in the current method mobile phase was used as diluent which makes a quick column saturation and no further disturbance in the equilibrium between the analytes and stationary phase. The linearity established in the previous method, for DEX and SER were 0.6-9.0 µg/mL and 2.8-42.0 µg/mL respectively, which was quite narrow, which makes the method less suitable to work with easy. The retention times and total run time was also less compared to previous method. Whereas the current method was developed with a wide range of linearity, for DEX and SER were 17.50 – 52.50 µg/mL and 90.00-270.00 µg/mL for SER. The previous methods robustness was also reported only by changing the flow rate (±0.1 mL/min) in the current method we studied robustness at three different conditions (flow rate: ±0.2 mL/min; temperature: ±5 °C and wavelength: °C 2 nm) and found the current developed method was robust at these conditions. We studied forced degradation too which proves that the method was stability indicating method.

Comparison with existing procedure is presented in Table 5. This method was proved to be fast, precise, selective, robust, and easy, and it may be used to a recently FDA authorized SER and DEX drug combination. This type of analysis may be used to determine the drug's safety, effectiveness, and quality in a cost-effective way. The developed technique was validated in accordance with ICH guidelines, and a stability studies showed that the approach was effective in monitoring drug stability. It may also be used for regular analysis in bioanalytical laboratories, hospital research institutes, quality control divisions of pharmaceutical companies, formulation dissolution studies, and accredited testing laboratories.

Acknowledgment

Authors are thankful to spectrum labs, Hyderabad for providing the APIs.

Table 5. Comparison of the developed method with existing method.

Parameter	Existing method [9]		Current method	
	DEX	SER	DEX	SER
Retention times (min)	4.258	5.62	4.60	2.39
Total run time (min)	10.00		6.00	
Accuracy (mean % recovery)	100.30	99.70	99.79	99.62
Precision (%RSD)	0.54	0.55	0.67	0.53
Linearity range (μg/mL)	0.6-9.0	2.8-42	17.50-52.50	90-270
LOD (μg/mL)	8.0	35.0	1.18	6.35
LOQ (μg/mL)	25.0	116.0	3.93	21.17

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