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Development and validation of novel rapid stability indicating mass compatible UPLC method for the simultaneous estimation of assay and related substances of oxytocin bulk and assay of injectable dosage form

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***Abstract*---**Developed and validated a simple, rapid, new mass compatible reverse phase ultra-performance liquid chromatography (UPLC) method for the simultaneous estimation of Assay and related substance of Oxytocin in bulk and extending the same method to injectable dosage form for the estimation of Assay. The developed method is capable of separating all the potential process and degradation impurities within 15 minutes. Chromatography was carried out using Waters Acquity CSH C18 column (50 mm length, 2.1 mm internal diameter and 1.7µ particle size) with gradient elution. 0.1% Trifluoroacetic acid used as Mobile phase A and mixture of 0.1% Trifluoroacetic acid and Acetonitrile in the ratio of 50:50 v/v was used as Mobile phase B. The flow rate of the mobile phase was set at 0.4 mL/min and the column temperature was maintained at 35°C. The detection was carried out using UV detector at 220 nm. The Retention time of Oxytocin is about 3 minutes and all the potential impurities are eluted within 10 minutes. Method validation was performed according to ICH (International Conference on Harmonization) guidelines. The validated method is simple, rapid, mass

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compatible, economical, specific, accurate, linear, precise, rugged, sensitive, robust and stability indicating. This method can be employed for the simultaneous estimation of assay and related substance of Oxytocin bulk and assay of injectable dosage forms at quality control laboratories of pharmaceutical industries.

***Keywords*---**oxytocin, method development, method validation, stability indicating, assay, related substances, UPLC.

Introduction

Oxytocin is a synthetic nonapeptide hormone used to induce or strengthen uterine contractions in pregnant women to aid in labor and delivery or to control postpartum bleeding [1]. Oxytocin is one of two neurohormones released by the posterior lobe of the pituitary. It is available in the form of Injectable dosage form. It can be administered by intramuscular injection or intravenous infusion of dilute solution. It should never be given by rapid intravenous bolus. If hemorrhage is risk, more active oxytocic agents such as ergots may be needed[2]. It is said and proved to produce many important physiological actions. Oxytocin when present in optimum level helps to maintain emotional homeostasis, pleasure, love, orgasm and also improves social memory and cognition. Deficiency of the same at the time of labour leads to uterine inertia and postpartum bleeding[3]. Any oxytocic drug administered in the third stage of labour reduces the blood loss with approximately 40% and hence the incidence of post-partum haemorrhage redcues from 10 to 6%[4]. An intranasal administration of oxytocin, a neuropeptide that plays a key role in social attachment and affiliation in non-human mammals, causes a substantial increase in trust among humans, thereby greatly increasing the benefits from social interactions [5]. In experimentally induced myocardial infarction in rats, continuous in vivo Oxytocin delivery improves cardiac healing and cardiac work, reduces inflammation, and stimulates angiogenesis [6]. Oxytocin repertoire has expanded to maintain a central role in more complicated aspects of reproductive behavior. For these reasons, Oxytocin called as the great facilitator of life [7]. It has a Molecular formula $C_{43}H_{66}N_{12}O_{12}S_2$ with a molecular weight of 1007.19 g/mol. The structure is presented in Figure 1. The literature survey revealed that there are research publications for estimation of assay of Oxytocin in bulk and pharmaceutical dosage forms by HPLC[8-12], stability indicating assay and related substances of Oxytocin bulk by HPLC[13], estimation of Oxytocin in diluting solutions like 0.9% sodium chloride, 5% dextrose and ringer lactate solution by HPLC[14-16], estimation of Oxytocin in biological solutions[17-19] and estimation of Oxytocin from milk by HPLC[20]. There are few LC-MS methods are available for estimation of Oxytocin and identification of degradation products[21-23], estimation of Oxytocin in rat and human plasma[24], saliva[25-26] and extraction and quantification of Oxytocin from Milk[27-28]. The USP[29] and EP[30] monographs are available for estimation of Oxytocin and related substances. In these monographs, 2 separate methods are followed for estimation of assay and related substances. These methods are longer run time, no known impurities are listed in the monograph, few known impurities are co-eluting and not compatible with LCMS analysis as it contains non-volatile buffers. Further review of literature indicates a lack of UPLC method that can quantify Oxytocin and

its potential related substances accurately using ultra performance liquid chromatographic procedure. UPLC procedures are short and help quick analysis of the samples that is key to providing access of the medicines to the patients in a timely manner. The delay in analysis due to separate assay and related substances method coupled with longer run time for related substances method causes delay in release of product leading to shortage of drug product in market leading to loss of precious human lives. Hence, this research work was carried out to minimize the product release time by reducing the analysis time by developing a short and cost-effective test procedure. As per the literature search, to the best of our knowledge, there is no stability indicating UPLC method for simultaneous estimation of assay and related substances of Oxytocin from bulk and pharmaceutical dosage forms in public domain. Hence, an attempt made to develop a simple, rapid, mass compatible stability indicating UPLC method for simultaneous estimation of assay and related substances from bulk and pharmaceutical dosage forms and validate the developed method according to ICH guidelines for validation of analytical procedures: Text and methodology Q2(R1) [31-33].

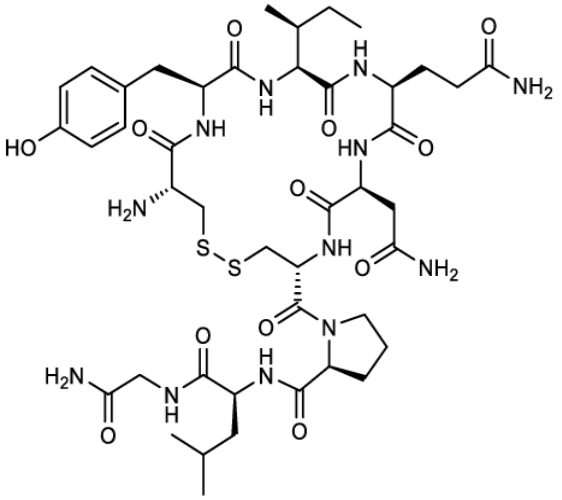

Figure 1: Structure of Oxytocin

Materials

Chemicals and Reagents

HPLC grade Trifluoroacetic acid, Acetonitrile and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water from Milli-Q water purification system was used throughout the study. Commercially available Oxytocin Injection drug product was used for this study and Oxytocin, Impurities were procured from contract laboratories.

Instruments

Waters Acquity UPLC High class separation module equipped with Photodiode Array Detector/TUV detector was used. Empower software was used for data acquisition and processing.

Analytical method development and methods

Screening of mobile phase, columns, diluent and sample concentration

The reverse phase UPLC method was developed by varying different chromatographic conditions including buffer, pH, mobile phase ratio, gradient, solvents, columns, flow rate, column oven temperature and diluent based on established method development strategies [32-33]. The mobile phase and stationary phase (Column) were selected based on physico chemical properties of Oxytocin. The pKa of Oxytocin is 9.5 (Strongest acidic) and 7.65 (Strongest basic) with a logP value of -5.0. Based on the pKa value, acidic mobile phase with 0.1% Trifluoroacetic acid was selected to have sharp peak with consistent retention time, and it is suitable for LC-MS analysis. Oxytocin and few of its impurities are close resembling structure and having different polarities. The method was optimized to separate all the known impurities from Oxytocin and each other by using gradient mode separation with smaller dimension column (1.7µ particle size and 50 mm column length) in order to have a shorter run time. The optimized method showed good separation of impurities, Oxytocin and its impurities were separated within 10 minutes with resolution of more than 2.0 for closest eluting impurities. Oxytocin is soluble in water and no degradation was observed when the solution is stored at refrigerated condition for more than 2 days. Hence, water finalized as diluent. The drug product is having very low concentration of Oxytocin (5 IU/mL = 8.3 µg/mL of Oxytocin and 10 IU/mL = 16.6 µg/mL of Oxytocin) hence injected as such as neat injection to have adequate response in UV detection. The sample concentration of Oxytocin bulk drug and injection volume was optimized to get a LOQ of 0.05%.

Methodology

Mobile phase A: 0.1% Trifluoroacetic acid, filtered through 0.22µ membrane filter.
Mobile phase B: Degassed mixture of 0.1% Trifluoroacetic acid buffer and Acetonitrile in the ratio of 50:50 v/v respectively.
Diluent: Water used as diluent.

Chromatographic condition

Column: Waters Acquity CSH C18 (50 mm x 2.1 mm, 1.7µ)
Flow rate: 0.4 mL/min
Column oven temperature: 35°C
Auto sampler temperature: 5°C
Injection Volume: 3 µL
Wavelength of detection: 220 nm.
Sampling rate: 5 pts/sec
Data acquisition time: 12 minutes
Next injection delay: 3 minutes
Gradient program:

Time (Minutes)	Mobile phase A (%)	Mobile phase B (%)	Curve
0	70	30	Initial

0.5	70	30	6
8.0	65	35	6
12.0	60	40	6
12.1	70	30	6
15.0	70	30	6

Preparation of standard solution

Accurately weighed 20 mg of Oxytocin reference standard into a 20 mL volumetric flask, dissolved and diluted to volume with diluent and mixed well to get a concentration of 1 mg/mL of Oxytocin.

Preparation of sensitivity solution

Pipetted out 100 µL of the 1 mg/mL standard solution into a 100 mL volumetric flask, diluted to volume with diluent and mixed well to get a concentration of 1 µg/mL of Oxytocin.

Preparation of sample solution for Assay and Related substance of bulk drug

Accurately weighed 20 mg of Oxytocin sample into a 20 mL volumetric flask, dissolved and diluted to volume with diluent and mixed well to get a concentration of 1 mg/mL of Oxytocin.

Preparation of sample solution for Assay from injectable dosage form

Injected neat injection without any dilution for Oxytocin Injection 5 IU/mL and 10 IU/mL. One IU(International unit) is equal to 1.666 µg of Oxytocin.

System suitability criteria

Blank in single, Sensitivity solution in single, standard solution in 5 replicate injections. The signal to noise ratio (S/N) for Oxytocin peak in sensitivity solution should be not less than 10. The % RSD for the peak area of Oxytocin from five replicate standard injections should be not more than 0.73%, theoretical plates should be not less than 2,000 and tailing factor should be not more than 2.0. Duplicate injections of sample solution were made upon successful demonstration of system suitability.

Analytical method validation

The analytical method validation for the simultaneous estimation of Assay and related substance of Oxytocin in bulk was conducted in accordance with ICH Guidelines [31] and demonstrated its suitability for the injectable dosage form for the estimation of assay. Analytical method validation parameters such as system suitability, specificity (Matrix interference and forced degradation), precision (Method precision and Intermediate precision), linearity, accuracy, limit of detection (LOD), limit of quantitation (LOQ), solution stability and robustness were performed according to ICH guidelines [31] to demonstrate the suitability of the

method for the simultaneous estimation of assay and related substances of Oxytocin.

System suitability

System suitability was demonstrated by injecting 3 µL of sensitivity solution in single, standard solution in 5 replicates prior to injecting sample solutions for each validation parameters. The system suitability results are presented in Table 1.

Table 1: System suitability results

System suitability parameter	Acceptance Criteria	Results	Pass/Fail
S/N ratio of Oxytocin from Sensitivity solution	NLT 10	15.5	Pass
% RSD for peak area of Oxytocin	NMT 0.73%	0.1	Pass
Tailing factor for Oxytocin	NMT 2.0	1.9	Pass
Theoretical plates for Oxytocin	NLT 2000	3971	Pass
% RSD for RT of Oxytocin	NMT 1.0%	0.2	Pass

Specificity

The specificity of the analytical method was evaluated by injecting blank, placebo, individual known impurities and sample spiked with all the known impurities at 1% level to demonstrate that the method has the ability to quantify the analyte in the presence of components which may be expected to be present e.g., degradation products and excipients [31]. The results are provided in Table 2 and Sensitivity, Spiked sample chromatograms are presented in Figure 2 and 3 respectively.

Table 2: Specificity results

Name	Common name	RT(Min)	RRT	Resolution	Peak purity		
					Purity Angle	Purity Threshold	Results
Impurity-1	Leu-3-Oxytocin	2.835	0.910	-	1.764	1.977	Pass
Oxytocin	Oxytocin	3.113	1.000	1.7	0.209	0.305	Pass
Impurity-2	Oxytocic acid	3.650	1.172	3.1	1.848	2.073	Pass
Impurity-3	Asp-5-Oxytocin	4.061	1.304	2.6	2.082	2.395	Pass
Impurity-4	Glu-4-Oxytocin	4.425	1.421	2.3	2.044	2.308	Pass
Impurity-5	Ala-3-CN-5-Oxytocin	5.659	1.818	7.3	2.014	2.337	Pass
Impurity-6	Ac-Oxytocin	7.114	2.28	7.4	1.823	2.126	Pass

Impurity-7	Parallel Dimer	8.307	5 2.668	5.9	2.337	2.667	Pass
Impurity-8	Anti parallel dimer-1	9.864	3.168	8.2	2.042	2.368	Pass
Impurity-9	Anti parallel dimer-2	10.301	3.309	2.3	2.414	2.705	Pass

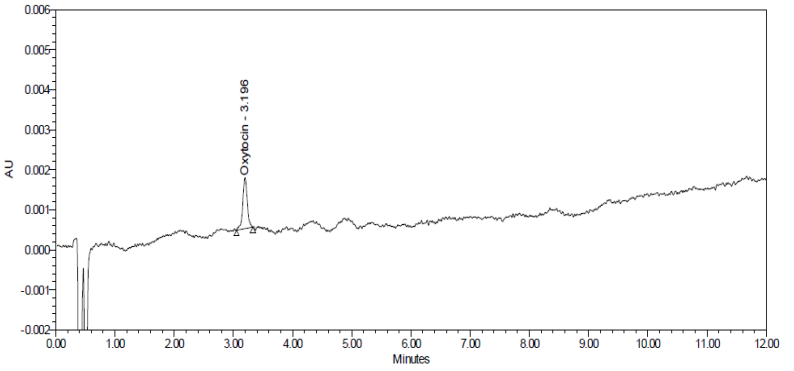

Figure 2: Chromatogram of sensitivity solution

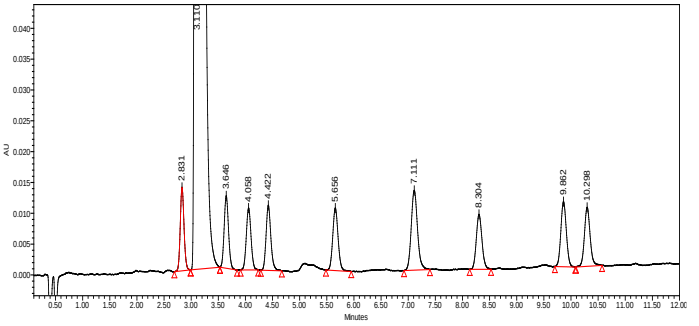

Figure 3: Chromatogram of Oxytocin sample spiked with known impurities

Forced degradation studies

Drug substance was subjected to acid, base, peroxide, thermal and photolytic stress conditions to induce deliberate degradation of drug substance. Degraded samples were injected into a UPLC equipped with PDA detector. Stability indicating nature of the method was proved by assessing the peak purity data of Oxytocin in stressed samples. Forced degradation results are provided in Table 3.

Table 3: Forced degradation results

Name of the stress sample	Peak purity of Remdesivir peak			Assay (%)	Total degradation (%)	Mass balance (%)
	Purity Angle	Purity Threshold	Results			
Sample as such (Unstressed)	0.166	0.304	Pass	100.1	1.53	N/A
Acid degradation	0.116	0.296	Pass	88.8	11.08	98.3

Name of the stress sample	Peak purity of Remdesivir peak			Assay (%)	Total degradation (%)	Mass balance (%)
	Purity Angle	Purity Threshold	Results			
Base degradation	0.141	0.301	Pass	90.8	8.05	97.3
Oxidation	0.158	0.298	Pass	99.6	1.38	99.4
Thermal degradation	0.154	0.309	Pass	96.1	1.83	96.4
Photolytic degradation	0.180	0.313	Pass	99.8	1.51	99.7
Water hydrolysis	0.143	0.298	Pass	100.3	1.42	100.1

Method precision/Repeatability

Precision (repeatability) was demonstrated by analyzing six replicate preparations of Oxytocin drug substance (bulk) and Oxytocin Injection 5 IU/mL and 10 IU/mL drug product. The assay and related substances of 6 replicate sample preparations are calculated and results are provided in Table-4 and 5 respectively for bulk and injectable dosage form.

Intermediate precision/ Ruggedness/Reproducibility

Intermediate precision was performed by analyzing six replicate preparations of Oxytocin drug substance (bulk) and Oxytocin Injection 5 IU/mL and 10 IU/mL drug product by different UPLC, different column on different days. The % assay, % related substance, SD, % RSD for 12 replicate preparations (n=6 for method precision and n=6 for intermediate precision) are calculated and results are provided in Table-4 and 5 respectively for bulk and injectable dosage form.

Table 4: Comparison of Method precision and Intermediate precision for Bulk drug

Validation parameter	No. Replicates	% Assay	Results in %					
			Impurity-2	Impurity-4	Impurity-6	Impurity-7	RRT 1.64	Total impurities
Method precision	Set-1	99.8	0.10	0.06	0.69	0.08	0.29	1.22
	Set-2	99.7	0.10	0.07	0.69	0.08	0.28	1.22
	Set-3	99.9	0.11	0.06	0.71	0.10	0.29	1.27
	Set-4	99.5	0.10	0.06	0.67	0.09	0.31	1.23
	Set-5	100.9	0.09	0.06	0.70	0.07	0.30	1.22
	Set-6	99.4	0.09	0.07	0.68	0.10	0.31	1.25
Intermediate precision	Set-1	100.8	0.08	0.07	0.71	0.07	0.32	1.25
	Set-2	101.0	0.08	0.06	0.68	0.08	0.29	1.19
	Set-3	101.1	0.09	0.08	0.66	0.08	0.30	1.21
	Set-4	101.4	0.08	0.08	0.67	0.06	0.27	1.16
	Set-5	101.0	0.09	0.08	0.64	0.09	0.27	1.17
	Set-6	101.3	0.08	0.08	0.70	0.06	0.42	1.33
Mean (n=12)		100.5	0.09	0.07	0.68	0.08	0.30	1.23

SD (n=12)	0.7542	0.0100	0.0090	0.0210	0.0151	0.0396	0.0458
% RSD (n=12)	0.8	11.1	12.9	3.1	16.1	13.2	3.7

Table 5: Comparison of Method precision and Intermediate precision for Injectable dosage form

Name	Assay of Oxytocin Injection 5 IU/mL		Assay of Oxytocin Injection 10 IU/mL	
	Method Precision	Intermediate Precision	Method Precision	Intermediate Precision
Set-1	101.7	104.2	101.4	106.9
Set-2	103.1	108.1	103.6	103.6
Set-3	103.8	105.8	102.5	105.3
Set-4	104.6	107.7	104.0	104.4
Set-5	102.5	106.3	102.4	105.0
Set-6	103.0	104.0	103.1	105.8
Mean (n=6)	103.1	106.0	102.8	105.2
SD (n=6)	1.0	1.7	0.9	1.1
% RSD(n=6)	1.0	1.6	0.9	1.1
Overall Mean (n=12)	104.6		104.0	
Overall SD (n=12)	2.0		1.6	
% Overall RSD(n=12)	1.9		1.5	

Accuracy

Accuracy is closeness of obtained value with true value or an accepted reference value [31]. The accuracy of the analytical method for assay was demonstrated from linearity experiments in the range of 20% to 120% of nominal test concentration. The accuracy for the related substances method was demonstrated by spiking all the known impurities in sample from LOQ to 200% of the nominal specification limit of 1.0%. The amount added, amount recovered, % recovery, % mean recovery and % relative standard deviation was calculated at each level. Mean recovery at each level is provided in Table 6 and 7 for both assay and RS method.

Table 6: Accuracy for Related substance:

Accuracy for RS Method								
Accuracy level in % of spec limit	Mean % Recovery (n=3 at each level)							
	Impurity -1	Impurity -2	Impurity -3	Impurity -4	Impurity -5	Impurity -6	Impurity -7	Impurity -8
LOQ	97.9	105.3	101.5	102.9	99.5	92.8	91.2	100.9
50	100.8	95.8	99.5	100.5	104.4	97	99.9	102.5
100	102.5	100.6	101.7	104.1	103.5	104.1	102.4	102.7
200	100	100.4	100.2	102.4	101.8	102.8	105.2	103.7
Overall Mean	100.3	100.5	100.7	102.5	102.3	99.2	99.7	102.5

recovery								
STDEV	1.91	3.88	1.05	1.5	2.16	5.25	6.05	1.16
% RSD	1.9	3.86	1.04	1.46	2.11	5.29	6.07	1.13

Table 7: Accuracy for Assay:

S.No	Accuracy level w.r.to Test concentration (%)	Concentration added (µg/mL)	Area Response (AU)	Recovered Concentration (µg/mL)	% Recovery	Overall Mean % Recovery
1	20	201.863	1456716	200.542	99.3	
2	50	504.656	3700982	508.373	100.7	
3	80	807.450	5792355	795.232	98.5	99.7
4	100	1009.313	7226266	991.912	98.3	
5	120	1211.175	8978949	1232.316	101.7	
Correlation Coefficient	0.999					
Regression Coefficient	0.999					
Intercept	-50829.90714					
Slope	7339.600556					

Linearity

Linearity of the analytical method was evaluated by plotting peak area and concentration of Oxytocin in the range of LOQ to 120% of the nominal test concentration and LOQ to 200% of nominal specification limit of 1.0% to demonstrate that the absorbance is directly proportional to the concentration of analyte in the sample [31]. Correlation coefficient, regression coefficient, intercept and slope are calculated from the regression line. Results are provided in Table 8 for Oxytocin and Table 9 to 10 for impurities. Linearity graph for Oxytocin is presented in Figure-4.

Table 8: Linearity for Oxytocin

S.No	Linearity level w.r.to Test concentration (%)	Concentration (µg/mL)	Area Response (AU)
1	0.05	0.505	3351
2	0.1	1.009	7647
3	0.2	2.019	13073
4	0.5	5.047	37053
5	1.0	10.093	70535
6	2	20.186	143900
7	5	50.466	373732
8	10	100.931	739325
9	20	201.863	1456716
10	50	504.656	3700982

11	80	807.450	5792355
12	100	1009.313	7226266
13	120	1211.175	8978949
	Correlation Coefficient		1.000
	Regression Coefficient		1.000
	Intercept		-5350.45673
	Slope		7290.583716

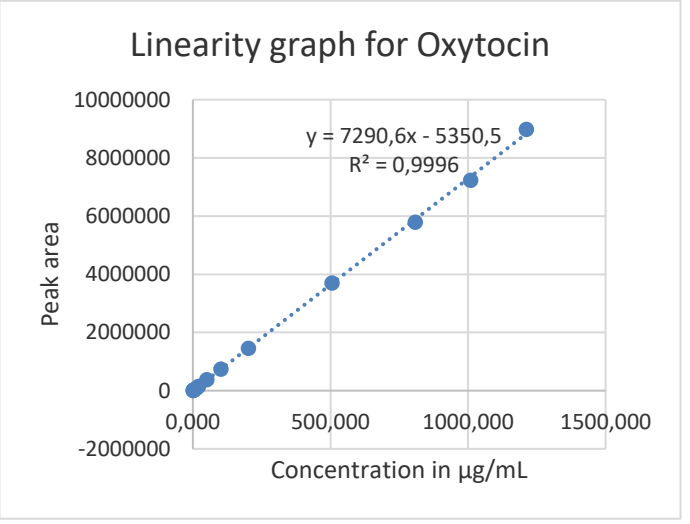

Figure 4: Linearity graph for Oxytocin

Table 9: Linearity for Impurities 1 to 4

S. No	Linearity level	Impurity-1		Impurity-2		Impurity-3		Impurity-4	
		Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
1	LOQ	0.501	2703	0.500	2864	0.507	2393	0.503	2681
2	10	1.001	5608	1.001	6475	1.014	6037	1.007	7228
3	20	2.002	12221	2.002	12839	2.027	11929	2.013	11077
4	50	5.005	31824	5.004	29864	5.069	30386	5.033	29300
5	80	8.008	51986	8.006	47096	8.110	47842	8.052	45408
6	100	10.011	63406	10.008	58753	10.137	55779	10.066	57352
7	150	12.013	77782	12.009	73027	12.164	68345	12.079	69224
8	200	15.016	95698	15.011	88788	15.206	89712	15.098	87812
Correlation Coefficient		1.000		1.000		0.999		1.000	
Regression Coefficient		1.000		1.000		0.999		1.000	
Intercept		-343.4462		429.9717		-130.2981		254.5063	
Slope		6410.226		5901.733		5787.439		5710.341	

Table 10: Linearity for Impurities 5 to 8

S. No	Linearity	Impurity-5	Impurity-6	Impurity-7	Impurity-8

	level	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area	Conc. (µg/mL)	Peak area
1	LOQ	0.505	4241	0.487	1267	0.504	2239	0.501	3718
2	10	1.010	5750	0.975	5542	1.007	3719	1.001	6537
3	20	2.019	12063	1.949	10775	2.014	9708	2.003	12776
4	50	5.048	31768	4.874	28009	5.036	24553	5.006	33372
5	80	8.076	53634	7.798	48631	8.058	46376	8.010	57260
6	100	10.095	67543	9.747	60689	10.072	58927	10.013	70529
7	150	12.114	81660	11.697	73712	12.087	72008	12.015	88809
8	200	15.143	102497	14.621	91706	15.108	88065	15.019	110007
Correlation Coefficient		1.000		1.000		0.998		1.000	
Regression Coefficient		1.000		1.000		0.997		0.999	
Intercept		-776.0084		-1787.304		-1368.129		-1287.178	
Slope		6749.871		6424.105		5809.538		7326.098	

Relative response factor

The relative response factor of impurities with respect to Oxytocin was calculated from slope of regression line of impurities to slope of regression line of Oxytocin. The obtained relative response factor is between 0.8 to 1.2 for all the impurities indicating the response of impurities and Oxytocin are similar.

Range

The range of an analytical procedure is the interval between the lower and upper concentration of analyte with demonstrated precision, accuracy and linearity [31]. The range of the method is LOQ (0.05%) to 120% of nominal test concentration for Oxytocin and LOQ (0.05%) to 200% of specification limit for impurities.

Limit of Detection (LOD) & Limit of Quantitation (LOQ)

LOQ was demonstrated at reporting threshold (0.05%) and LOD at 0.03% for Oxytocin and its all the known impurities. Signal to noise ratio(S/N) was calculated for all the peaks. The results of LOD and LOQ are provided in Table 11.

Table 11: LOD and LOQ Results

Name	S/N of LOD(0.03%) (Limit: ≥ 3)	S/N of LOQ(0.05%) (Limit: ≥ 10)
Impurity-1	9.3	22.7
Oxytocin	7.8	25.5
Impurity-2	7.5	17.3
Impurity-3	7.6	15.6
Impurity-4	5.5	15.2
Impurity-5	9.7	17.9
Impurity-6	3.0	10.5
Impurity-7	6.3	12.8

Impurity-8	4.5	14.2

Solution stability

The standard and sample solutions were injected at different interval up to 48 hours by keeping the solution at 5°C. After 48 Hrs, Standard solution area differed from initial by 1.9% and sample by 0.5% and solutions are considered stable for 48 Hrs at 5°C.

Robustness

Robustness of the analytical method was investigated under a variety of conditions by making deliberate changes in chromatographic parameters. During robustness study effect of composition of buffer and acetonitrile in the mobile phase B (50:50 ± 1% absolute), flow rate (0.40± 0.05 mL/min), column temperature (35±5°C) and TFA buffer concentration (± 0.01% absolute) was evaluated. The system suitability criteria of signal to noise ratio, %RSD, tailing factor and theoretical plates were evaluated in all the robustness conditions. Spiked sample was injected in each robustness condition and resolution between impurities was evaluated.

Table 12: Robustness

Name of the condition	S/N of Sensitivity solution (Limit: NLT 10)	% RSD (Limit: NMT 0.73%)	Tailing factor (Limit: NMT 2.0)	Theoretical plates (Limit: NLT 2000)
Original condition (Control)	15.5	0.1	1.9	3971
Increase in flow rate (+0.05 mL/min)	21.0	0.2	1.9	3898
Decrease in flow rate (-0.05 mL/min)	18.0	0.1	1.9	3532
Increase in column oven temp (+5°C)	17.1	0.4	1.9	3454
Decrease in column oven temp (-5°C)	13.0	0.6	1.9	3855
Increase in Organic phase (+1%) in MP-A & B	28.4	0.04	1.3	2906
Decrease in Organic phase (-1%) in MP-A & B	14.3	0.04	1.2	3717
Increase in Buffer concentration (+0.01%)	13.9	0.01	1.9	3478
Decrease in Buffer concentration (-0.01%)	20.4	0.04	1.9	3348

Discussions

A novel stability indicating RP-UPLC method was developed and subjected to validation challenges in accordance to ICH guideline [31]. The system suitability results met the predefined acceptance criteria throughout the study. No interference was observed at the retention time of Oxytocin from blank and impurities. All the known impurities were found well separated from Oxytocin and each other with a resolution greater than 2.0 between any closest pairs. Peak purity was found passing for Oxytocin peak in spiked sample and in all the forced degradation condition samples confirming that no impurity co-elutes with Oxytocin peak. Oxytocin degraded in acidic and basic stress conditions predominantly forming Impurity-2, Impurity-3, Impurity-4 and Impurity-7. All the degradants are well separated from Oxytocin peak and each other indicating the method is specific and stability indicating. Therefore, this method is suitable for stability analysis of Oxytocin. The RSD for the six replicate assay results from method precision experiment found to be 0.8% and RSD for total impurities are 3.7% indicating the method is precise for the determination of assay and related substances. The overall %RSD for the assay and impurities from 12 results (n=6 from method precision and n=6 from intermediate precision) found to be 0.8% and 3.7% respectively for bulk drug and less than 2.0% for assay of injectable dosage form indicating the method is precise, repeatable, rugged and reproducible when analyzed on different days using different column and UPLC.

The % accuracy of Oxytocin at each level calculated from regression line were ranging from 98.3% to 101.7% for assay with overall mean accuracy of 99.7%. The % overall mean recoveries of impurities (Impurity 1 to 8) are found to be in the range of 99.2% to 102.5% which is well within the generally acceptable range of 85% to 115% indicating that the method is accurate in the range of LOQ to 200% of specification limit of impurities and 20% to 120% of test concentration for assay. The method is linear from 0.5 µg/mL to 1200 µg/mL for Oxytocin. Linearity demonstrated from LOQ (0.05%) to 200% of specification limit for impurities. The correlation coefficient of regression line was found to be 1.000 for Oxytocin and greater than 0.995 for all the known impurities indicating that the method shows excellent linearity in the above stated range. The response factor of impurities is comparable with Oxytocin therefore impurities can be quantified by area normalization without applying nay response factors. Limit of detection and limit of quantification was demonstrated to be 0.03%w/w and 0.05%w/w respectively for Oxytocin and its impurities. The signal to noise ratio for Oxytocin and all the known impurities in LOD, LOQ solutions are found to be greater than 3 and 10 respectively indicating that the method is highly sensitive to detect and quantify the presence of very low concentrations of impurities in the sample. The predefined system suitability criteria were met, and all the impurities are well separated from Oxytocin and each other for all the robustness conditions indicating that the deliberate change in the chromatographic parameters has no significant effect on chromatographic behavior of the method, therefore the method is robust. The standard and sample solutions are found to be stable for 48 hours at refrigerated temperature. The range of the method is defined as LOQ (0.05%) to 120% of nominal test concentration for Oxytocin and LOQ to 200% of specification limit for impurities based on demonstrated linearity, accuracy and precision.

Conclusion

A novel stability indicating, mass compatible UPLC method has been developed for the simultaneous estimation of Assay and related substance of Oxytocin in bulk and demonstrated the suitability of this method for the estimation of assay of Oxytocin in injectable dosage form. The developed method is capable of separating all the potential impurities within 15 minutes with superior resolution capabilities. The developed method is demonstrated to be simple, fast, economic, mass compatible, specific, stability indicating, accurate, linear, precise, rugged, sensitive, robust and high throughput. The same method can be extended for content uniformity of finished products. Hence, this method can be employed for the estimation of assay, content uniformity and related substances in bulk and finished products stability studies in quality control laboratories of pharmaceutical industries to enhance the access of the drug product with shorter time period. The developed method if employed in Quality control laboratory has the potential to significantly reduce the drug substance, drug product release time and aid in saving of precious human lives.

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