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Research Article

A NOVEL STABILITY-INDICATING RP-UPLC METHOD FOR THE QUANTIFICATION OF IMPURITIES AND A NEW QDa MASS DETECTOR COUPLED WITH LC-PDA FOR IDENTIFICATION OF MASS OF DEGRADATION PRODUCTS IN A FIXED DOSE COMBINATION OF EMPAGLIFLOZIN AND LANIGLIPTIN TABLETS USED AS SECOND-LINE THERAPY FOR TYPE-2 DIABETES

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ABSTRACT

A new UPLC method was developed for identification and quantification of process related impurities and degradation products in a new fixed dose combination product of empagliflozin and linagliptin tablets, which is used to treat type-2 diabetes. Chromatographic separation was obtained using a new phenomenex Luna omega polar C18, 100x2.1mm, 1.6µ and a gradient programme consisting of Mobile phase A: 10mM Potassium dihydrogen orthophosphate pH-3.0 and Mobile phase B: acetonitrile and methanol (55:45%v/v). Degradation impurities were monitored at a common wavelength of 225nm. The run time was 40 minutes within this run time the five related compounds, all major degradation impurities of empagliflozin and linagliptin were eluted which reduces the analysis time and solvent consumption. The combined drugs as well as individual blends were subjected to hydrolysis (water, acid and base), oxidative, photolytic and thermal stress conditions. Mass of major unknown degradation products were determined by LC-PDA coupled with a new QDA mass detector. The protanated molecular ion peaks for linagliptin at M+H were DP1-514.19 in water, acid, base, oxidative, photolytic and thermal stress conditions. DP2-515.14 in acid and base hydrolysis. DP3-544.18 in acid hydrolysis. For empagliflozin DP4-365.13 in base hydrolysis. DP5-487.16 and DP6-487.14 in peroxide hydrolysis. DP7-470.91, DP8-502.08, DP9-538.01, DP10-326.98 and DP11-293.02 in acid hydrolysis. The developed method was validated as per international conference on harmonization guidelines (ICH) with respect to specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and robustness.

Key words: UPLC. QDa mass detector. Method development. Empagliflozin and linagliptin. Stress degradation products. Validation.

INTRODUCTION

The chemical name of empagliflozin is (EPG; (2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol. Empagliflozin is the sodium-dependent glucose co transporter 2 (SGLT-2), it reabsorbs the renal glucose [1-3]. Chemical name of linagliptin is (LNG; 1H-Purine-2, 6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1-yl)-3, 7-dihydro-3-methyl-1-[(4-methyl-2quinazolinyl) methyl]. Linagliptin is an inhibitor of the enzyme dipeptidyl peptidase 4 (DPP-4) used for the treatment of diabetes mellitus type 2 [4-7]. Glyxambi is a fixed dose combination (FDC) product containing two drug substances, empagliflozin and linagliptin. It is a first combination product which has dual mechanism on type-2 diabetes. Two dosage strengths are available for empagliflozin (10mg or 25mg) and a fixed amount of linagliptin (5mg). Empagliflozin is a SGLT2 inhibitor and linagliptin is a DPP-4 inhibitor. SGLT2 is a protein that re absorbs the glucose from the kidney into the blood. Empagliflozin inhibits SLGT2 and lowers blood glucose levels and increase glucose excretion. DPP-4 enzyme cleaves into glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP)-1. GIP is secreted from K cells of the upper intestine and GLP-1 is secreted from L cells of the lower intestine [8-10].

Literature survey reveals that a variety of chromatographic methods and stability indicating methods have been reported for the determination of empagliflozin and linagliptin in empagliflozin and linagliptin tablets by HPLC [11,12], empagliflozin and metformin in empagliflozin and metformin by HPLC [13,14]. A few stability indicating methods available for the determination and quantification of empagliflozin and linagliptin by UPLC [15]. Few reports were also available for the determination of empagliflozin [16,17] and linagliptin [18,19] alone by HPLC. Very few bio-analytical methods available for the determination of empagliflozin and linagliptin by LCMS/MS [20-24]. Hence decided to develop a new stability indicating method for the quantification of related compounds of empagliflozin and linagliptin in fixed dose combination of empagliflozin and linagliptin tablets. Run time is very crucial and hence UPLC has been selected for this study. A new QDa mass detector was employed for the first time to determine the mass of degradation products formed stress studies.

The aim of this study is to develop a single, less run time and cost effective UPLC [25,26,27] method that could separate empagliflozin, linagliptin (Figure 1), their process and potential impurities namely EMP.RC01, EMP.RC02, EMP.RC03, LIN.RC01, LIN.RC02 (Figure 2) and also unknown degradation impurities during acid, base, peroxide, thermal [28] and photolytic [29] stress conditions studies [30,31,32,33] and shelf life [34] of a product and identification of their mass by using a new QDa mass detector [35,36,37]. The method was validated according current ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

Reference standards, chemicals, impurities and finished product used for experiments were obtained from Hetero labs limited Hyderabad. Potassium dihydrogen orthophosphate from Merck, Orthophosphoric acid was purchased from Merck, Darmstadt, Germany, HPLC-grade acetonitrile and methanol from Rankem chemicals India. Purified water: distilled and deionized with the MILLIQ Gradient system (Pall, Washington, NY, USA).

Instrumentation

Waters UPLC® system (Waters Corp., Milford, MA, USA) equipped with an auto sampler and quaternary gradient pump with an in-line degasser was used. The photodiode array (PDA) detector was engaged throughout the analysis. The chromatographic data was acquired using Empower 3 software. LC/PDA/QDa experiments were carried out using Waters UPLC® system (Waters Corp., Milford, MA, USA) equipped with an auto sampler and quaternary gradient pump with an in-line degasser was used. The photodiode array (PDA) detector and quadrupole dalton (QDa) mass detector were utilized for degradation studies. Photostability studies were carried using Neutronic Photostability Chamber (Neutronic lifecare equipment Pvt. Ltd., India). The thermal degradation studies were carried in the Neutronic laboratory oven (Neutronic lifecare equipment Pvt. Ltd., India). Ultrasonic bath (PCi analytics, India) was used to dissolve the samples and pico pH meter (LABINDIA, analytical, India) was used to adjust the pH of the buffer for mobile phase.

Chromatographic conditions

A Luna Omega polar C18 100x2.1mm, 1.6µ column using Mobile phase A with Potassium dihydrogen orthophosphate (10mM), pH-3.0 and Mobile phase B was acetonitrile and methanol in the ratio of 55:45%v/v. The gradient program T (min) =% mobile phase B: 0=20, 5=35, 15=35, 20=60, 25=60, 30=65, 35=75, 35.5=20 and 40=20 with flow rate 0.5 mL/min. 225nm has been finalized based on the response of two main components and all other impurities. A typical chromatogram was recorded at this wavelength. The injection volume was 2µL. The column temperature was maintained at 35°C. Sample cooler was at 5°C. Acetonitrile and methanol in the ratio of 50:50%v/v is used as diluent.

LC-QDa mass detector conditions

The LC–QDa detector (Waters HPLC empower-3 software with Acquity QDa detector (Mass detector)) was used for the identification of known and unknown related compounds formed during forced degradation studies. A Phenomenex kinetex C18, 100 x 4.6 mm, 2.6-µ column is used as the stationary phase with a mobile phase containing solvent A 0.1% (1mL/L) formic acid in water and solvent B (Acetonitrile and Methanol (80:20%v/v)). The flow rate of the mobile phase was kept at 0.6 mL/min at run time of 60 minutes with a gradient program of 0/25, 7/40, 25/60, 30/70, 40/80, 50/80, 51/25 and 60/25 (time (min)/%B). The injection volume was 10µL. The column temperature was maintained at 35°C. Wavelength scanning was from 200nm to 400nm for detector1 (PDA detector). Ionization mode was ESI+, probe temperature was 450°C, capillary voltage was 0.8kv, acquisition type was full scan m/z 100 to 1250 (centroid) and cone voltage was 15 volts for detector2(QDa detector). The column temperature was maintained at 35°C.

Preparation of solutions

Preparation of standard solution

A standard stock solution of empagliflozin (1250µg/mL) and linagliptin (250µg/mL) were prepared in appropriate proportion of empagliflozin and linagliptin working standard in diluent. A standard solution containing 6.25µg/mL of empagliflozin and 1.25µg/mL of linagliptin was prepared from standard stock solution.

Preparation of sample solution

A test solution containing 1250µg/mL of empagliflozin and 250µg/mL of linagliptin was prepared by taking tablet powder equivalent to 25mg of linagliptin dissolved in 100 mL of 50:50%v/v of acetonitrile and methanol, sonicated sample solution for 30 minutes. Centrifuged the sample solution at 5000 rpm for 5 minutes. Filtered the sample solution through 0.22µ PVDF (Millipor) filter. The concentration (125µg/mL of empagliflozin and 25µg/mL of linagliptin) of above sample solution was diluted to 10 times and determined for assay.

Preparation of spiked sample solution

Stock solutions of impurities (100µg/mL) were prepared in 50:50%v/v of acetonitrile and methanol. A sample solution containing 1250µg/mL of empagliflozin and 250µg/mL of linagliptin was spiked with five impurities at 0.5% level was used as spiked sample (Figure 3).

Method validation

The described method has been validated for the related compounds by UPLC determination. According to the FDA and ICH guidelines[38]. The key analytical parameters that are required for validation are specificity, precision, accuracy, linearity, LOD, LOQ, ruggedness and robustness.

Specificity

The ability of the method that could be well resolved from all its degradation impurities, process impurities and unknown degradation impurities formed during degradation studies. Placebo should not be interference at the retention time of active as well as impurities. Specificity was confirmed by injecting individual impurities at specification level and also from spiked sample preparation spiked with all impurities at specification level.

Forced degradation studies

Forced degradation studies were established to provide an indication of the stability indicating property and specificity of the proposed method. Unconventional stressed conditions were applied for both tablets and active pharmaceutical ingredient (API) to study the sensitivity of the drug product and drug alone. Stress studies were employed on equivalent quantity of empagliflozin and linagliptin tablets by treating with acid (5M HCl, 60°C, 10min), base (5M NaOH, 60°C, 10 min), oxidative (10 % H2O2, 60°C, 10 min), thermal (80 °C, 24 hrs), and photolytic (overall illumination not less than 1.2 million lux hours, and integrated near ultraviolet energy not less than 200 W h/m2) and empagliflozin blend alone subjected to (5M HCl, 60°C, 24hrs), base (5M NaOH, 60°C, 24hrs), oxidative (30 % H2O2, 60°C, 24hrs), thermal (80°C, 24 hrs), and photolytic (overall illumination not less than 1.2 million lux hours, and integrated

near ultraviolet energy not less than 200 W h/m2) with an initial concentration of 1250µg/mL of empagliflozin and 250µg/mL of linagliptin was exposed to prove the proposed method to separate empagliflozin and linagliptin from its degradation products. Stress chromatograms were illustrated in Fig 4(a)-(i). Homogeneity of empagliflozin and linagliptin peaks in stressed samples ensured by peak purity. Photo diode array was employed throughout the analysis to assess the peak purity. QDa mass detector was used to determine the mass of degradation products.

Precision

System precision for the proposed method was confirmed by injecting a standard solution containing Empagliflozin and linagliptin at 0.5% of specification level (1250µg/mL of empagliflozin and 250µg/mL of linagliptin) six times into the chromatographic system and reported %RSD. The repeatability of the method was demonstrated for six sample preparations containing 1250µg/mL of empagliflozin and 250µg/mL of linagliptin tablets spiked with 0.5% of all five impurities and reported %RSD for all impurities. Intermediate precision was also demonstrated by preparing standard solution preparation and spiked sample preparation same as above on different day, different analyst, different instrument and reported %RSD.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

A series of solutions containing empagliflozin and linagliptin and all its five impurities was used to established LOD and LOQ at a signal to noise ratio of 3:1 and 10:1 respectively. Precision at LOQ was also established using above sample solutions and reported %RSD for all peaks.

Linearity

The linearity of detector response for empagliflozin, linagliptin and their all five impurities were determined by preparing a series of solutions having empagliflozin, linagliptin and its five impurities at five different concentrations levels ranging from 0.05% to 150% of test concentration (1250µg/mL for empagliflozin and 250µg/mL for linagliptin). The correlation coefficients, slopes, R^2 value and *y*-intercepts of the calibration curves were reported.

Accuracy

The accuracy of an analytical procedure expresses the closeness of results between true value and the value found. The study was carried out by spiking known impurities in triplicate at 0.05%, 100% and 150% of the analyte concentration (1250µg/mL for empagliflozin and 250µg/mL for linagliptin) and reported % recovery and %RSD at each level.

Robustness

The robustness of an analytical procedure is capacity of the method remain unaffected by small, deliberate changes in method conditions, it provides positive indication of the method during normal use. To demonstrate robustness, the experimental conditions were altered and resolution of empagliflozin, linagliptin and its impurities was evaluated. To study the effect of the flow rate on resolution, the flow rate was changed from 0.45 and 0.55 mL min−1. The outcome of the column temperature was studied at 30 and 45 °C. The effect of buffer pH was studied by varying ±0.2 pH units (at 2.8 and 3.2 buffer pH).

Table 1: System suitability results

Compound	RT(%RSD)[a]	RT (in min)[a]	RRT[b] (n=6)	Peak area (%RS D)[a] (n=6)	USP tailing[a] (n=6)	Resolution[c]
LIN RC02	0.1	6.117	0.93	0.5	1.0	---
Linagliptin	0.0	6.588	---	1.3	1.1	5.3
EMP RC01	0.0	7.123	0.45	3.5	1.1	5.3
EMP RC02	0.0	10.765	0.68	1.5	1.0	26.0
Empagliflozin	0.0	15.758	---	1.2	1.1	20.5
LIN RC01	0.0	19.352	2.94	2.9	1.0	16
EMP RC03	0.1	26.825	1.70	1.5	1.0	59.4

[a] Mean (n= 6). [b] Relative retention times (RRT)were calculated against the retention time (RT) of Empagliflozin and Linagliptin, [c] Resolutions were calculated between two adjacent components.

Table 2: Summary of forced degradation studies

Parameter	Empagliflozin and Linagliptin tablets % of impurity formed								
	Empagliflozin			Linagliptin		% Assay		Mass balance	
	EMP RC01	EMP RC02	EMP RC03	LIN RC01	LIN RC02	EMP	LIN	EMP	LIN
Acid (5N,60°C,10min)	ND	ND	ND	ND	ND	99.6	59.0	100	95.8
Base (5N,60°C,10min)	ND	ND	ND	ND	ND	98.9	43.4	100	96.8
Peroxide (10%,60°C,10min)	ND	ND	ND	ND	ND	98.5	90.2	100	97.5
Water (10%,60°C,10min)	ND	ND	ND	ND	ND	99.3	98.7	100	99.5
Thermal 105°C/24hrs	ND	ND	ND	ND	ND	99.1	97.8	100	98.0
Photolytic	ND	ND	ND	ND	ND	98.9	97.5	100	98.2
Empagliflozin blend									
Acid (5N,60°C,10min)	ND	0.31	ND	ND	ND	98.0	ND	95.3	ND
Base (5N,60°C,10min)	ND	ND	ND	ND	ND	99.8	ND	98.2	ND
Peroxide (10%,60°C,10min)	1.59	ND	ND	ND	ND	93.6	ND	98.3	ND

Table 3: Summary of mass results

Active name	Degradation condition	Name of degradation product	Relative Retention time (RRT)	Mass of degradation product (M+H)
Linagliptin	Base	DP1	0.96[a]	365.13
	Acid	DP2	1.03[a]	544.18
	In all conditions	DP3	1.15[a]	514.19
	Acid and Base	DP4	2.55[a]	515.14
	Peroxide	DP5	2.69[a]	487.16
		DP6	2.79[a]	487.14
Empagliflozin	Acid	DP7	0.10[b]	326.98
		DP8	0.44[b]	293.02
		DP9	0.96[b]	470.91
		DP10	1.25[b]	502.08
		DP11	1.32[b]	538.01

[a] Relative retention time with respect to Linagliptin and impurities belongs to Linagliptin
[b] Relative retention time with respect to Empagliflozin and impurities belongs to Empagliflozin

Table 4: LOD, LOD, Regression and precision data

Parameter	EMP[a]	LIN[b]	Empagliflozin			Linagliptin	
			EMP RC01	EMP RC02	EMP RC03	LIN RC01	LIN RC02
LOD ($\mu g\ mL^{-1}$)	0.213	0.042	0.218	0.210	0.215	0.042	0.041
LOQ ($\mu g\ mL^{-1}$)	0.641	0.127	0.653	0.624	0.645	0.126	0.123
Precision (%RSD) at LOQ	3.2	4.3	1.8	2.6	3.1	5.2	6.4
Signal/ Noise	22.94	20.91	26.08	11.20	44.51	12.06	11.55
Regression equation(Y) Slope(b)	3987.93	3014.51	2665.10	2189.22	924.01	5020.37	4746.24
Intercept(a)	-28.09	181.23	-120.65	-235.28	122.62	267.32	21.46
Correlation coefficient	1.0	0.9958	0.9991	0.9986	0.9980	0.9998	0.9910
R^2 Value	1.0	0.991	0.998	0.996	0.996	0.999	0.990
Precision (%RSD)	1.3	1.2	3.4	1.5	1.5	2.9	0.5
Intermediate Precision (%RSD)	1.7	1.9	2.3	1.2	1.7	1.1	1.6

[a] Empagliflozin, [b] Linagliptin

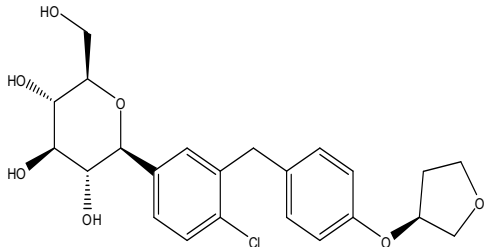
Table 5: Evaluation of accuracy

Amount spiked[a]	% Recovery[b]				
	Empagliflozin			Linagliptin	
	EMP RC01	EMP RC02	EMP RC03	LIN RC01	LIN RC02
0.05%	95.8	96.6	96.7	99.8	101.4
(%RSD)	0.47	0.41	0.53	1.74	0.86
100%	95.8	96.3	95.6	104.8	103.3
(%RSD)	0.36	1.74	0.53	0.29	0.92
150%	96.1	96.0	94.1	100.9	101.6
(%RSD)	0.45	0.69	0.12	3.39	1.19

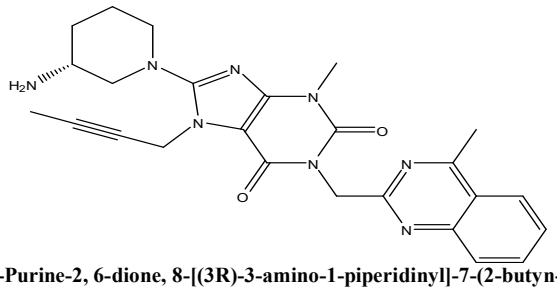
[a] Amount of all impurities spiked individually from 0.05% to 150%, [b] Mean, %RSD for three determinations

Table 6: Robustness data

Robustness condition	LIN RC-02	Linagliptin	EMP RC-01	EMP RC-02	Empagliflozin	LIN RC-01	EMP RC-03
Resolution							
Temperature 30°C	-	5.2	6.0	26.3	23.8	16.9	60.8
Temperature 35°C	-	5.3	5.3	26.0	20.5	16.0	59.4
Temperature 40°C	-	4.7	4.8	25.1	20.3	20.2	57.5
pH-2.8	-	5.3	5.4	25.8	20.4	18.5	60.2
pH-3.0	-	5.3	5.3	26	20.5	16	59.4
pH-3.2	-	4.9	5.1	26.6	20.7	16.4	59.6
Flow-0.4ml/min	-	3.6	4.6	25.5	21.6	16.3	60.5
Flow-0.5ml/min	-	5.3	5.3	26.0	20.5	16.0	59.4
Flow-0.6ml/min	-	5.6	5.4	26.6	20.6	17.6	62.7

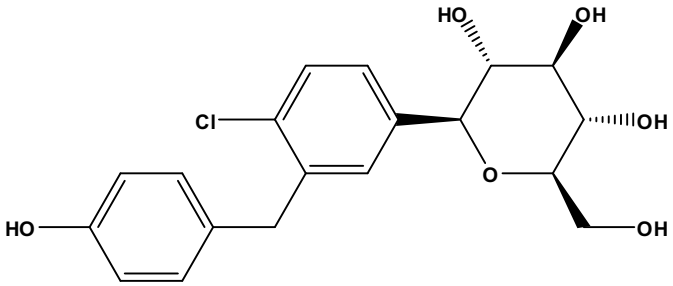


(2S,3R,4R,5S,6R)-2-[4-chloro-3-[[4-[(3S)-oxolan-3-yl]oxyphenyl]methyl]phenyl]-6-(hydroxymethyl)oxane-3,4,5-triol
Empagliflozin

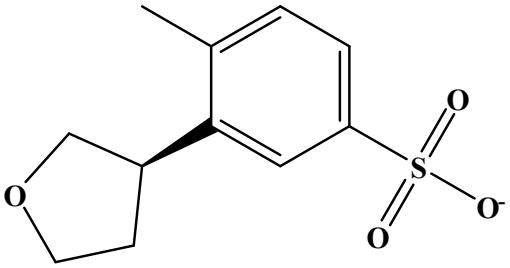


1H-Purine-2, 6-dione, 8-[(3R)-3-amino-1-piperidinyl]-7-(2-butyn-1-yl)-3, 7-dihydro-3-methyl-1-[(4-methyl-2quinazolinyl) methyl]
Linagliptin

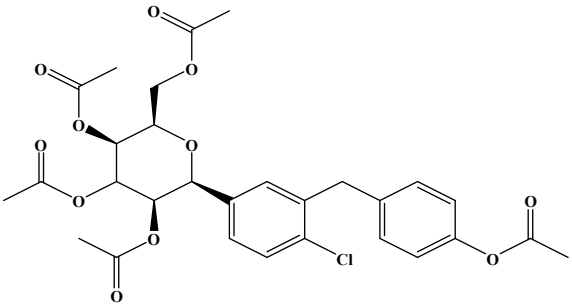
Figure 1



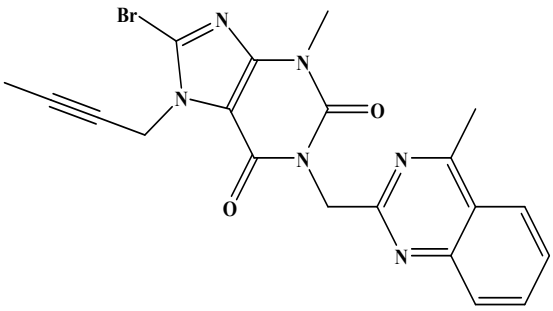
(2S,3R,4R,5S,6R)-2-(4-chloro-3-(4-hydroxybenzyl)phenyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol
EMP RC-01



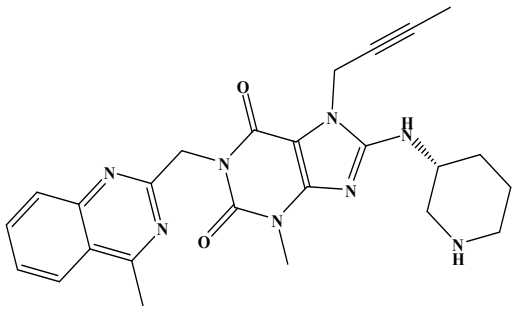
3(R)-Tetrahydro-3-furanyl-4-methylbenzenesulfonate
EMP RC-02



(2S,3R,4R,5S,6R)-2-(3-(4-acetoxybenzyl)-4-chlorophenyl)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate
EMP RC-03



8-Bromo-7-(but-2-ynyl)3-methyl-1-((4-methyl quinazolin-2-yl)methyl)-1H-purine-2,6(3H,7H)-dione
LIN RC-01



(R)-7-(but-2-yn-1yl)-3-methyl-1-((4-methylquinazolin-2-yl)methyl)-8-(piperidin-3-ylamino)-3,7-dihydro-1H-purine-2,6-dione
LIN RC-02

Figure 2

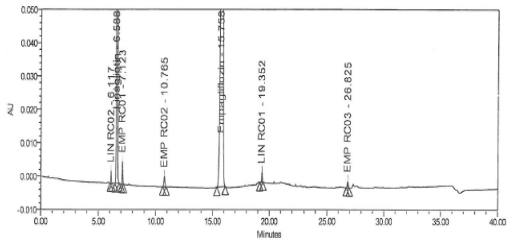
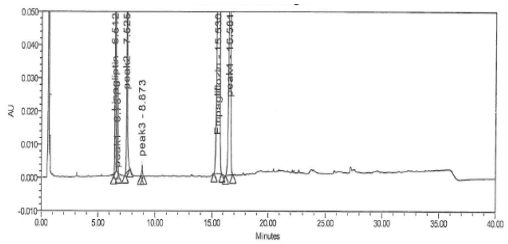
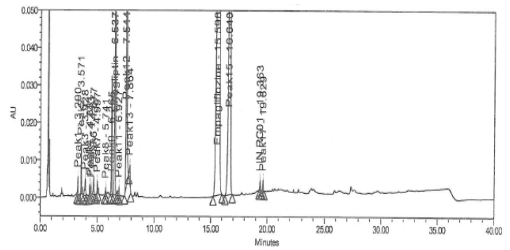


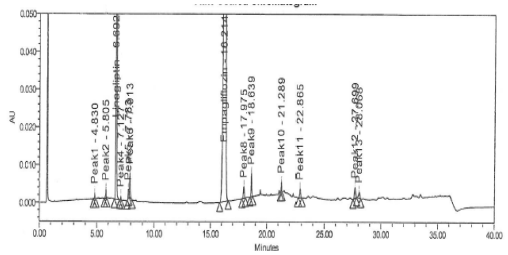
Figure 3: Typical chromatogram of spiked sample preparation



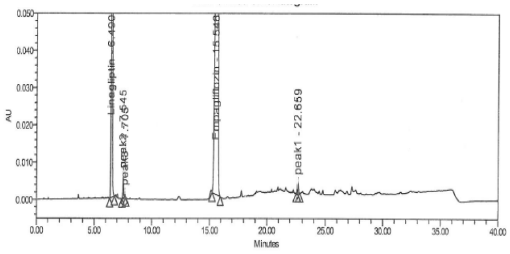
(a) Chromatogram of sample under acid stress condition



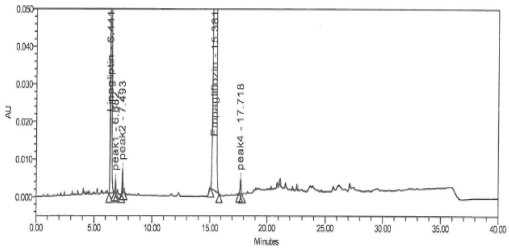
(b) Chromatogram of sample under base stress condition



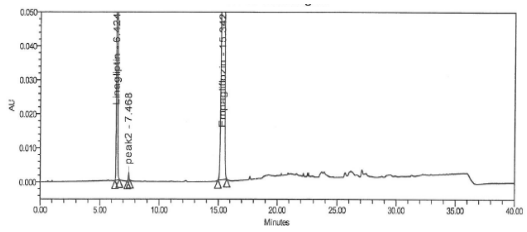
(c) Chromatogram of sample under peroxide stress condition



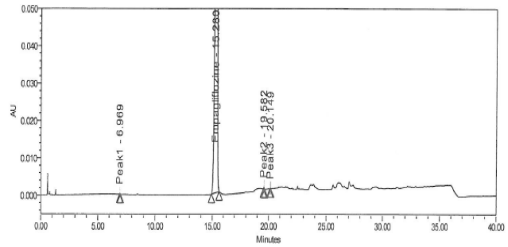
(d) Chromatogram of sample under thermal stress condition



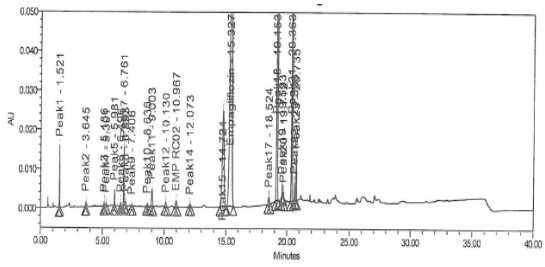
(e) Chromatogram of sample under photolytic stress condition



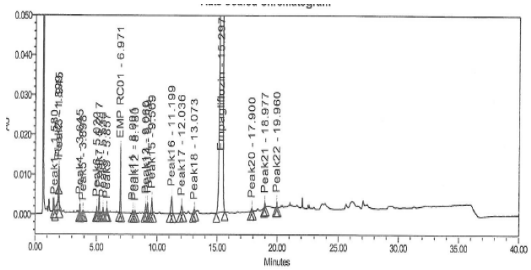
(f) Chromatogram of sample under water stress condition



(g) Chromatogram of Empagliflozin blend under base stress condition

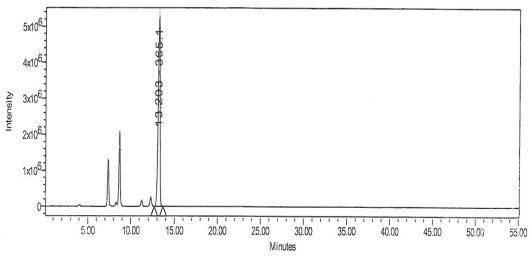


(h) Chromatogram of Empagliflozin blend under acid stress condition

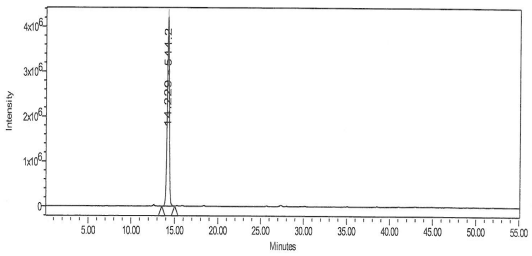


(i) Chromatogram of Empagliflozin blend under peroxide stress condition

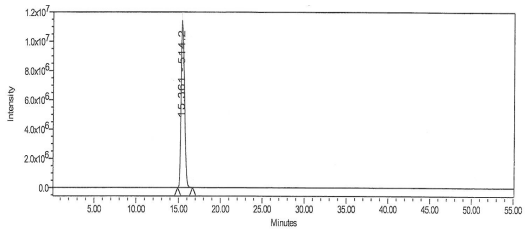
Figure 4



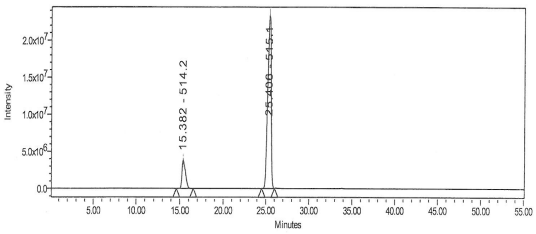
(a) Mass Spectrum of unknown degradant of Linagliptin under base stress condition



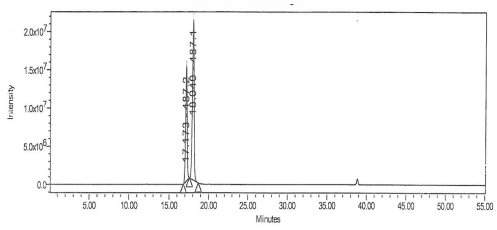
(b) Mass Spectrum of unknown degradant of Linagliptin under acid stress condition



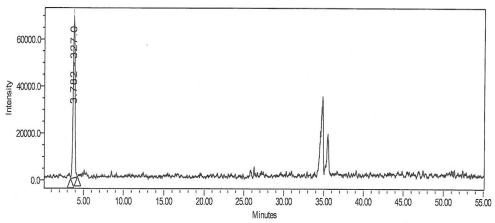
(c) Mass Spectrum of unknown degradant of Linagliptin in all stress conditions



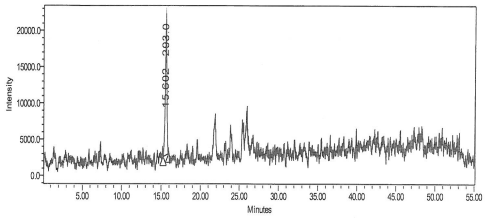
(d) Mass Spectrum of unknown degradant of Linagliptin in acid and base stress conditions



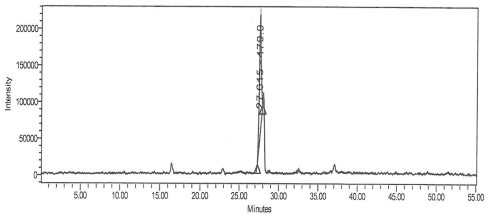
(e) Mass Spectrum of unknown degradants of Empagliflozin in oxidative stress conditions



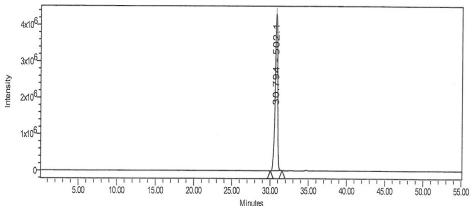
(f) Mass Spectrum of unknown degradants of Empagliflozin in acid stress conditions



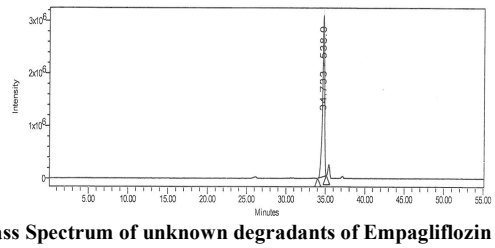
(g) Mass Spectrum of unknown degradants of Empagliflozin acid stress conditions



(h) Mass Spectrum of unknown degradants of Empagliflozin in acid stress conditions



(i) Mass Spectrum of unknown degradants of Empagliflozin in acid stress conditions



(j) Mass Spectrum of unknown degradants of Empagliflozin in acid stress conditions

Figure 5

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

The chromatographic method should separate impurities (key RAW materials, intermediates, bi-products from the synthesis of empagliflozin and linagliptin, stability impurities, and degradation products) and the main two components in a single UPLC method and it should be stability indicating, free of interference from excipients, robust and cost effective for routine use in quality control laboratory.

Selection of wavelength is very critical for different compounds with different absorptions maxima and especially for combination products. Empagliflozin, linagliptin and its impurities exhibit different maxima. But based in the response of individual components, impurities and also considering the lowest label claim of the drug (empagliflozin and linagliptin 10mg/5mg and 25mg/5mg) 225nm was opted as detection wavelength for the method. This wavelength is selective and sufficient for all impurities.

The affinity towards stationary is different for empagliflozin, linagliptin and its impurities due to different molecular structures and polarities. Different experiments were carried out using acquity BEH C18, acuity BEH C8, acuity BEH phenyl and phenomenex Luna omega polar C18. Better resolution, peak tailing was bringing in to finally on a new phenomenex Luna omega polar C18. Column temperature was maintained at 35°C for extended column life and repeatability of the method. The outcome of various temperatures on resolution was studied at 30°C, 35°C and 40°C. The impact of buffer pH on retention times of empagliflozin, linagliptin and their impurities were studied between pH, 2.0, pH 2.8 to pH 3.2, pH 5.0 and pH 7.0 keeping chromatographic parameters unaltered. A robust pH which did not have impact in resolution was fixed at pH 3.0. Different flow rates between 0.4 mL/min to 0.6mL/min were evaluated for all two active peaks and impurities. Finally flow rate was selected as 0.5ml/min based on the column back pressure, resolution between active as well as all impurities and run time.

After a series of experiments, the method has been concluded on phenomenex Luna omega polar C18 with 100 x 2.1mm, 1.6µ column using Mobile phase A with 10mM potassium dihydrogen orthophosphate, adjusted pH 3.0 with dilute orthophosphoric acid solution and Mobile phase B with acetonitrile and methanol in the ratio of 55:45 %v/v. The UPLC gradient programme T(min) =% mobile phase B: 0=20, 5=35,15=35,20=60, 25=60, 30=65 , 35=75, 35.5=20 and 40=20with flow rate 0.5 mL/min. Better base line and resolution between impurities was noticed at a column oven temperature of 35°C. A chromatogram obtained from empagliflozin and linagliptin spiked with their five impurities at 0.5% level was shown in Figure 3. The system suitability results were illustrated in Table 1

Results of forced degradation studies

Formation of EMP.RC01 was observed in oxidative and EMP.RC02 was found in acid stress conditions for empagliflozin alone. Three major unknown degradants in acid, one unknown major degradant in base stressed and one unknown degradant in peroxide stressed condition were observed for linagliptin in empagliflozin and linagliptin tablets. Five major unknown degradants were observed in acid stress conditions for empagliflozin alone. Various degradation products observed in acid, base, peroxide, thermal and photolytic conditions were represented in Figure 4(a, b, c, d, e, f, g, h,i). Mass balance, % assay and % of impurities formed during stress conditions were reported in Table 2. The major unknown degradation products formed in the above stress conditions were identified by PDA detector and their mass by a new QDA mass detector. The protonated molecular ion peaks for linagliptin degradation products were at M+H DP1-514.19 in water, acid, base, oxidative, photolytic and thermal stress conditions. DP2-514.14 in acid and base hydrolysis. DP3-544.18 in acid hydrolysis. DP4-365.13 in base hydrolysis. DP5-487.16 and DP6-487.14 in peroxide hydrolysis. For empagliflozin at M+H DP7-326.98, DP8-293.02, DP9-470.91, DP10-502.08 and DP11-538.01 in acid hydrolysis. Mass spectrum of unknown degradation products formed during degradation studies were represented in Figure 5(a, b, c, d, e, f, g, h, i, j). Mass of unknown degradation peaks were illustrated in Table 3.

Results of method validation

Precision

The repeatability of the method was demonstrated for six sample preparations containing 1250µg/mL of empagliflozin and 250µg/mL of linagliptin tablets spiked with 0.5% of all five impurities Fig. 2. The %RSD for the area of each peak was calculated and found to be within 3.4 %. The intermediate precision of the method was also demonstrated using different analyst, different instrument and performing the analysis on different days. The %RSD for the area of each peak was also calculated and found to be within 2.3%. The results were tabulated in Table 4.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Decreasing concentrations of standard and its five impurities were injected in the above said chromatographic conditions for the determination of LOD and LOQ. The LOD and LOQ of the method were determined by injecting standard solutions of progressively decreasing concentration under the chromatographic conditions described above. The LOD was explained as the lowest concentration for which the signal-to-noise ratio was 3:1 and the LOQ was defined as the lowest concentration for which the signal-to-noise ratio was 10:1. The established LOD and LOQ values for empagliflozin, linagliptin and its five impurities were tabulated in Table 4.

Linearity

The linearity of detector response for empagliflozin, linagliptin and their all five impurities were determined by preparing a series of solutions having empagliflozin, linagliptin and its five impurities at five different concentrations levels ranging from 0.05% to 150% of test concentration (1250µg/mL for empagliflozin and 250µg/mL for linagliptin). The correlation coefficients, slopes, R^2 value and *y*-intercepts of the calibration curves were calculated (Table 4).

Accuracy

The accuracy of an analytical procedure expresses the closeness of results between true value and the value found. The study was carried out by spiking known impurities in triplicate at %0.05%, 100% and 150% of the analyte concentration (1250µg/mL for empagliflozin and 250µg/mL for linagliptin). The % recoveries of all these impurities were found to be in-between the predefined acceptance criterion of 85.0-115.0%. The % assay of empagliflozin and linagliptin was ranging between 98.5 to 101.5% w/w
(Table 5).

Robustness

The robustness of an analytical procedure is capacity of the method remain unaffected by small, deliberate changes in method conditions, it provides positive indication of the method during normal use. To demonstrate robustness, the experimental conditions were altered and resolution of empagliflozin, linagliptin and its impurities was evaluated. To study the effect of the flow rate on resolution, the flow rate was changed from 0.45 and 0.55 mL min−1. The outcome of the column temperature was studied at 30 and 45 °C. The effect of buffer pH was studied by varying ±0.2 pH units (at 2.8 and 3.2 buffer pH) (Table 6).

Solution stability

Solution stability was carried out by keeping spiked sample solutions in sample cooler at 5°C for 48 h and at room temperature. Content of all five impurities were determined for every 12 h interval up to the study period. No remarkable changes were observed in the content of these impurities during the solution stability experiments. 100% organic solvent has been used as diluent for sample preparation to control solvent evaporation. Hence solution stability was established up to 48 hours at 5°C.

CONCLUSION

A simple, sensitive and a new stability-indicating, gradient RP-UPLC method has been developed for the quantitative determination of empagliflozin, linagliptin and its related impurities in drug product. This method can able to fit for separating all process impurities, known and unknown degradation products with good resolution within 40 min. Forced degradation studies were conducted, mass of major degradants were identified using QDA mass detector with RP-HPLC method. A systematic mechanism for the formation of degradation impurities was proposed based on the known reactivity of the drug through hydrolysis, thermal and photolytic. The behavior of empagliflozin, linagliptin under various stress conditions and identification of mass for unknown degradation products were studied and presented for the first time. The developed method was validated as per the ICH guidelines and found to be specific, precise, accurate and linear. The LOQ value reveals the sensitivity of the method which can put into use for quality control analysis of pharmaceutical dosage forms.

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REFERENCES

1. Empagliflozin as add-on therapy in patients with type 2 diabetes inadequately controlled with linagliptin and metformin; a 24-week randomized, double-blind, parallel-group trial, care. Diabetesjournals.org. 2016 40(2): 201-209.
2. Gangadharan Komala M, Mather A: Empagliflozin for the treatment of Type 2 diabetes. Expert Review of Clinical Pharmacology. 2014 7(3): 271-9.https://doi.org/10.1586/17512433.2014.908703 PMid:24716752
3. Lamos EM, Younk LM, Davis SN: Empagliflozin, a sodium glucose cotransporter 2 inhibitor, in the treatment of type 1 diabetes. Expert Opinion on investigational Drugs. 2014 23(6): 875-82.https://doi.org/10.1517/13543784.2014.909407 PMid:24746173
4. Deacon, C.F.; Holst, J.J. Linagliptin, a xanthine-based dipeptidyl peptidase-4 inhibitor with an unusual profile for the treatment of type 2 diabetes. Expert Opinion on investigational Drugs. 2010 1:133–140. https://doi.org/10.1517/13543780903463862 PMid:19947894
5. Guedes, E.P.; Hohl, A.; de Melo, T.G.; Lauand, F. Linagliptin: Pharmacology, efficacy and safety in type 2 diabetes treatment. Diabetology and metabalic. syndrome. 2013 5: 25.
6. Barnett, A.H. Linagliptin: A novel dipeptidyl peptidase 4 inhibitor with a unique place in therapy. Advances in therapy. 2011 28: 447–459. https://doi.org/10.1007/s12325-011-0028-y PMid:21603986
7. Del Prato, S.; Barnett, A.H.; Huisman, H.; Neubacher, D.; Woerle, H.J.; Dugi, K.A. Effect of Linagliptin monotherapy on glycaemic control and markers of β-cell function in patients with Inadequately controlled type 2 diabetes, A randomized controlled trial. Diabetes Obesity and metabolism. 2011 3: 258–267. https://doi.org/10.1111/j.1463-1326.2010.01350.x PMid:21205122
8. Andrew Lewin, Ralph A. DeFronzo, Sanjay Patel, Dacheng Liu,Renee Kaste, Hans J. Woerle, and Uli C. Broedl. Combination of Empagliflozin and Linagliptin as Second-Line Therapy in Subjects With Type 2 Diabetes Inadequately Controlled on Metformin. Diabetes Care. 2014 37(6):1650-1659.
9. Single-pill combination therapy for type 2 diabetes mellitus: linagliptin plus empagliflozin, Current Medical Research & Opinion, 2015.
10. Cost-effectiveness of second-line antihyperglycemic therapy in patients with type 2 diabetes mellitus inadequately controlled on metformin. Canadian medical association journal. 2011 183(16):1213-1220. https://doi.org/10.1503/cmaj.110178 PMid:21969406 PMCid:PMC3216433
11. Development and validation of stability indicating RP-HPLC method for simultaneous estimation of mpagliflozine and linagliptin in tablet formulation, Der Pharmacia Lettre. 2016 8 (17):57-65.
12. RPHPLC method development and validation for simultaneous determination of Linagliptin and Empagliflozin in tablet dosage form; An International Advanced Research Journal in Science, Engineering and Technology. 2015 2(2).
13. A new validated RP-HPLC method for the determination of Metformin HCl and Empagliflozin in its bulk and pharmaceutical dosage forms; International Journal of Pharmaceutical sciences and Research. 2016 8 (5):2223-32.
14. Development and validation of stability indicating RP-HPLC method for the simultaneous estimation of metformin HCl and Empagliflozin in bulk and in a synthetic mixture. International of pharmacy. 2016 6(4):138-147.
15. UPLC simultaneous determination of empagliflozin, linagliptin and metformin new combination. Royal society of chemistry. 2015 5: 95703-95709.
16. Method development and validation of empagliflozin by RP-HPLC in bulk and pharmaceutical dosage form, An International Journal of Advances in Pharmaceutical Sciences. 2016 7(1):3040-3042.
17. Development and validation of a novel stability-indicating RP-HPLC method for the determination of empagliflozin bulk and pharmaceutical dosage form; International Journal of Pharmaceutical sciences and Research. 2006 7(11):4523-30.
18. QbD Based Method Development on an Agilent 1290 Infinity UHPLC System Combined with a Seamless Method Transfer to HPLC Using Intelligent System Emulation Technology. 2015 : 5591- 5701.

19. Syed Lateef, Vinayak A. K. Stability Indicating Method Development for Linagliptin Drug Product using Quality-by-design, Agilent Technologies, publication number. 2014: 5591-3834.
20. Pharmacokinetic evaluation of Empagliflozin in healthy Egyptian volunteers using LC-MS/MS and comparison with ethnic populations, Scientific Reports. 2017 7(1): 2583. https://doi.org/10.1038/s41598-017-02895-7 PMid:28566743 PMCid:PMC5451423
21. LC-MS/MS determination of Empagliflozin and Metformin. Journal of chromatographic science. 2017 55(7):742-747. https://doi.org/10.1093/chromsci/bmx030 PMid:28383657
22. Bioanalytical method development and validation of linagliptin in plasma through LC-MS/MS. International journal of bioassays. 2014 3 (07):3146-3151.
23. Development and validation of LC-MS/MS method for simultaneous determination of Metformin and four gliptins in human plasma, Chromatographia. 2017 80: 891-899. https://doi.org/10.1007/s10337-017-3288-0
24. High throughput LC-MS/MS method for the quantitation of linagliptin in human plasma by solid extraction using phase 96 well plate format, International Journal of Pharmaceutical sciences and Research. 2016 7(3):1321-30.
25. Lanka A. Ramaprasad, J.V.L.N.S.Rao, Srinivasu Pamidi, Varaprasad J, Nagaraju D. Impurity profiling of Tolvaptan tablets by a stability indicating method by UPLC, International research journal of Pharmacy. 2012 3(11):145-149.
26. Kakumani Kishore Kumar, Chimalakonda Kameswara Rao, Maddala Vijaya Lakshmi, Khagga Mukkanti. A Validated Stability Indicating RP-UPLC Method for Atrovastain Calcium, American journal of analytical chemistry. 2012 3(5):392-399.
27. Lanka A.Rama Prasad, J.V.L.N.S.Rao, Srinivasu Pamidi, Vara Prasad. J, Kishore Kumar Hotha3, UPLC method for the determination of Eszopiclone and its related impurities, International Journal of Analytical and Bioanalytical Chemistry. 2012 2(4):241-246.
28. ICH Q1A (R2); Stability testing of new drug substances and products, 2003.
29. ICH Q1B Photostability Testing of New Drug Substances and Products, 1996.
30. Namdeo G. Shinde, Bhaskar N. Bangar , Sunil M. Deshmukh , Suyog P. Sulake , Dipak P. Sherekar, Pharmaceutical Forced Degradation Studies with Regulatory Consideration. Asian pharma press. 3 2013 (4):178-188.
31. Trivikram Rawat, I.P. Pandey, Forced degradation studies for Drug Substances and Drug Products- Scientific and Regulatory Considerations, Journal of pharmaceutical sciences and research. 2015 7(5):238-241.
32. Dan W. Reynolds, Kevin L. Facchine, June F. Mullaney, Karen M. Alsante, Todd D. Hatajik, andMichael G. Motto, Available Guidance and Best Practices for Conducting Forced Degradation Studies. Pharmaceutical technology. 2002:48-56.
33. R. Hajkova, P. Solich, J. Dvorák, J. Sicha, Simultaneous determination of methylparaben, propylparaben, hydrocortisone acetate and its degradation products in a topical cream by RP- HPLC. Journal of pharmaceutical and biomedical analysis. 2003 32:921-927. https://doi.org/ 10.1016/S0731-7085(03)00193-6
34. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Stability Testing, stability testing of New Drug Substances and Products, ICH Q1A, Geneva, Switzerland, 1998.
35. Margaret Maziarz, Mark Wrona, and Sean M. McCarthy Waters Corporation, Milford, MA, USA, Benefits of Using ACQUITY QDa Mass Detection for Quantitative Analysis of Non-Chromophoric Memantine HCl in Tablet Formulation, 2014.
36. Tracy Mui, Prabhakar Kasturi, Jinchuan Yang, Rich DeMuro and Joe Romano, Analysis of Flavonoids in Juices with the ACQUITY QDa Detector, 2014.
37. Mark Benvenuti, Dimple shah, and Jennifer A.Burgess, Waters Corporation, Milford, MA, USA, A Method for the Rapid and Simultaneous analysis of Sweeteners in various food products using the ACQUITY H-class System and ACQUIY QDa Mass detector, 2014.
38. ICH Q2 (R1), Validation of analytical procedures: text and methodology, 2005.

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