RESEARCH ARTICLE

STABILITY INDICATING UPLC METHOD FOR DETERMINATION OF IMPURITIES IN VERAPAMIL HYDROCHLORIDE DRUG SUBSTANCE

*,[1,2]Vijayabaskar, S., [1]Mahalingam, V. and [1]Kalaivani

[1]Department of Chemistry, Vel's Univeristy, Pallavaram, Chennai, India
[2]Research and Development Laboratory, Piramal Healthcare Ltd, Ennore, Chennai-600 057, India

ARTICLE INFO	ABSTRACT
	A novel, economic, and time-efficient stability-indicating, reverse-phase ultra-performance liquid chromatographic (RP-UPLC) method has been developed for the analysis of verapamil hydrochloride in the presence of both impurities and degradation products generated by forced degradation. When verapamil hydrochloride was subjected to acid hydrolysis, oxidative, base hydrolysis, photolytic, and thermal stress, degradation was observed only in oxidative and base hydrolysis. The drug was found to be stable to other stress conditions. Successful chromatographic separation of the drug from impurities formed during synthesis and from degradation products formed under stress conditions was achieved on a Shimpak XR ODS, 75 mm x 3.0mm, 1.7μ particle size column, UV detection at 278nm and a gradient elution of ammonium formate, orthophosphoric acid and acetonitrile as mobile phase. The method was validated for specificity, precision, linearity, accuracy, robustness and can be used in quality control during manufacture and for assessment of the stability samples of verapamil hydrochloride. To the best of our knowledge, a validated UPLC method which separates all the sixteen impurities disclosed in this investigation has not been published elsewhere. Total elution time was about 18 min which allowed quantification of more than 100 samples per day. The analytical method discussed in British Pharmacopeia was pH sensitive but the method reported in this study is not involved any pH adjustment.

INTRODUCTION

Verapamil hydrochloride, chemically α-(3-((2-(3,4-dimetho xyphenyl)ethyl)methylamino)-propyl)-3,4-dimethoxy- α-(1-methylethyl)benzene acetonitrile (British Pharmacopoeia, 2009) (The Merck Index, 14th Ed, 2006) is an oral and intravenous calcium-channel blocking (CCB) agent. It is useful for the treatment of angina, hypertension, and for supraventricular tachyarrhythmia. Verapamil hydrochloride is considered as a class IV antiarrhythmic agent and it is more effective than digoxin for controlling ventricular rate in patients with atrial fibrillation (Buzinkaiova *et al.*, 1995; Dethy *et al.*, 1994; Ewin, 2001; Il Farmaco, 2000; Gerard *et al.*, 2009) (Hardman *et al.*, 1999; Buzinkaiova *et al.*, 1995; Musshoff *et al.*, 2004; Martindale, 1996; Najma *et al.*, 2004). Verapamil hydrochloride has also been described as an inhibitor of P-glycoprotein which is able to improve the chemotherapy response by reducing the resistance of cancer cells against antineoplastic agents (Hardman, 1999; Warmann *et al.*, 2002). Recent studies on verapamil hydrochloride reveal, high-dose of

the drug is positive in the treatment of cluster headache. The dose of verapamil hydrochloride used for cluster headache is approximately double the dose used in cardiovascular disease. The effect of verapamil hydrochloride in cluster headache most likely takes place in the hypothalamus (International Conferences on Harmonization, 2006; International Conferences on Harmonization, 1994; Jhee *et al.*, 2005; Kasim *et al.*, 2002; Khalil and Kelzieh, 2002; Lednicer, 1995) (Peer *et al.*, 2009; Gerard *et al.*, 2009; Ewin 2001; Ozaltin *et al.*, 1997; Lew 1999; Lednicer, 1995). The different analytical techniques reported so far for the determination of this drug along with corresponding impurities are by capillary electrophoresis (Lew, 1999; Martindale, 1996) (Niveen *et al.*, 2000; Dethy *et al.*, 1994), spectrophotometry (Mohamed *et al.*, 2006; Musshoff *et al.*, 2004) (Nafisur *et al.*, 2005; Mohamed *et al.*, 2006), HPLC (Nafisur *et al.*, 2005; Najma and Tabassum, 2004; Niveen *et al.*, 2000; British Pharmacopoeia, 2009; Pauline *et al.*,1991; Jhee *et al.*, 2005), atomic emission spectrometry (Ozaltin and Kocer, 1997) (Khalil *et al.*, 2002) and electrochemistry method (Pauline *et al.*, 1991; Kasim *et al.*, 2002). This paper describes a simple linear gradient reverse phase UPLC method which separates all the sixteen impurities reported in British Pharmacopoeia (BP) (Nafisur *et al.*, 2005)

*Corresponding author: Vijayabaskar, S.
Research and Development Laboratory, Piramal Healthcare Ltd, Ennore, Chennai-600 057, India

and degradation products. The structure of verapamil hydrochloride and its impurities are illustrated in Figure 1. Organic impurities can arise during the manufacturing process and storage of the drug substances, the criteria for their acceptance up to certain limits are based on the pharmaceutical studies or known safety data defined in International Conferences on Harmonization (ICH) (Peer and Jacob, 2009) (ICH draft revised guidance on Impurities in New Drug Substances Q3A(R2).2006). Because a process for synthesis of verapamil hydrochloride has recently been developed in our laboratory, an RP-UPLC method was developed for the analysis of verapamil hydrochloride in the presence of its sixteen impurities. The analytical method discussed in BP (Nafisur *et al.*, 2005) was pH sensitive, but the method discussed in this study is pH independent. The accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness of the method were determined in accordance with ICH guidelines (The Merck Index, 2006) (International Conferences on Harmonization.Q2(R) Validation of Analytical Procedures.1994). This paper reports, for the first time a new, rapid, efficient, pH independent, simple and validated stability indicating UPLC method for separation of sixteen potential impurities and degradation products as 'one shot' analysis.

MATERIALS AND METHODS

Reagents and materials

Hydrochloric acid and sodium hydroxide were purchased from Damao Chemical Reagent Factory (Tianjin, China). Hydrogen peroxide (30%) was bought from Tianjin Fuyu Fine Chemistry Engineering Co., Ltd (Tianjin, China). Methanol and acetonitrile were HPLC grade and others were analytical grade. HPLC-grade water was purified by a Milli-Q Reagent Water system (Millipore, Bedford, MA) used in preparing the aqueous solutions and the mobile phase throughout the experiments. Sample of verapamil hydrochloride and its sixteen impurities A-P (Figure 1) were synthesized in our laboratory (address) and characterized by using MS, IR and NMR.

Instrumentation

Chromatographic analysis was performed on an ACQUITYTM UPLC system (Waters Corp., Milford, MA, USA), equipped with a binary pump solvent management system, micro degasser, an autoplate-sampler, and thermostatic column compartment. Chromatographic separation was carried out on a Shimpak XR ODS, 75 mm x 3.0mm, 1.7μ particle size column with an in-line filter (0.22 μm) prior to the column. DK-S26 water baths were equipped with MV controller, Electro-thermostatic blast oven (DHG-9146A, Shanghai Jinghong Experimental Equipment Co., Ltd. Shanghai, China), Hundred Thousandth Balance (AUW120D, SHIMADZU, Japan). A 50-W clear xenon lamp was employed as the light source for estimating the photolytic experiment (CEL-HXB F300, Beijing Zhongjiao Jinyuan technology co., Ltd. Beijing, China).

Stress degradation studies

Stress degradation studies of verapamil hydrochloride, PB-3 and DY-1 were carried out under hydrolysis (acid and base),

oxidation, photolytic and thermal forced conditions. The stock solutions of verapamil hydrochloride, PB-3, and DY-1 were prepared at the concentration of 0.2 mg·ml−1 by dissolving accurately weighted 10 milligrams of three analytes in 50 ml of methanol. One milliliters of these three stock solutions was taken precisely out 6 and put into a 10 ml volumetric flasks, respectively. The tests of acidic and basic hydrolysis were carried out in 2 ml of hydrochloric acid solution (1 mol·L -1) and sodium hydroxide solution (1 mol·L -1), respectively. The hydrolysis processes were conducted at 60℃ for 5 h and controlled the concentration of each analyte at 20 μg·mL-1 in a 10 ml volumetric flask. The oxidative degradation study was carried out in 2 ml of 30% of hydrogen peroxide at room temperature for 5 h and the concentration of each analyte was maintained at 20 μg·mL-1 in a 10 ml volumetric flask. In the tests of photolytic and thermal studies, five milligrams of verapamil hydrochloride, PB-3, and DY-1 were put on the watch glasses and kept at 150℃ for the thermal experiment and exposed to the light of 4000±500lux for the photolytic experiment for 12 h, respectively. The blank samples were prepared without adding the analytes in each stress condition.

Preparation of sample solutions

The solutions obtained in the acidic and basic hydrolysis tests were cooled down to room temperature and neutralized with sodium hydroxide solution (1 mol·L -1) and hydrochloric acid solution (1 mol·L -1), respectively. Then, they were diluted to the mark with acetonitrile. The solutions obtained in the test of oxidative degradation were diluted to the volume of 10 ml round bottom flask with acetonitrile, then diluted 20-fold with acetonitrile. The solutions obtained in the tests of photolytic and thermal degradation were diluted 200-fold with acetonitrile. Each sample was prepared in triplicate. All the solutions were filtered by 0.22 μm membrane filters and kept in refrigerator at 4℃ before UPLC analysis.

Chromatographic conditions

Analysis was performed on a Waters Acquity UPLC® equipped with diode array detector (DAD). Analysis was carried out at 278nm. Separation was achieved using Shimpak XR ODS fast LC column with sub2μ particle size, (75mm x 3.0 mm, 1.7μm particle size). The data was acquired via Waters Empower 2 software. Mobile phase-A was a mixture of 10mM ammonium formate and 1mL of orthophosphoric acid in 1L water. Mobile phase-B consists of a mixture of acetonitrile, water and orthophosphoric acid in the ratio 700:300:1. The gradient time program was (Tmin/A:B; T0.01/100:0; T8.0/60:40; T11.0/30:70; T17.0/10:90), thus the run time is 17.0 min. The initial eluent composition was restored at 18.0 min (100:0) and maintained further for 2.0 mins. The flow rate was set at 0.50mL min^{-1}, the column temperature was maintained at 50°C and the injection volume was 1.00μL. A mixture of water: acetonitrile (90:10) was used as a diluent for the preparation of standard and sample solutions. Both mobile phase and diluent were filtered through a nylon membrane filter (pore size 0.2μm).

A standard preparation consisting of 0.001mg/mL concentration of all impurities along with verapamil hydrochloride was prepared. A sample solution consisting of verapamil hydrochloride 1.0mg/mL was prepared.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Several LC methods with shorter run time and high throughput were tried for the separation of sixteen impurities along with verapamil hydrochloride. These includes different stationary phase, column dimension and buffers. Various trials and their conditions were given in Figure 2. Finally, the method was optimized with Shimpak XR ODS (75mm x 3.0 mm x 1.7μ particle size) column and initial mobile phase gradient condition of 100% solvents A, where A is mixture of 10mM ammonium formate and 0.1% OPA in water and B is 0.1% OPA in (70:30) (acetonitrile : water). The gradient time program was, initial (100 : 0), increased to (60:40) up to 8 min, and further altered to (30:70) up to 11.0 min. and further increased to (10:90) upto 17.0 min. Thus the run time is 17.0 min. The initial eluent composition was restored at 18.0 min and maintained for 2.0 mins. The flow rate was maintained at 0.50mL/min, the column temperature was maintained at 50°C and the injection volume was 1.00μL. Sample cooler is kept at 5°C. A mixture of water: acetonitrile (90:10) was used as a diluent for the preparation of standard and sample solutions. All the impurities and verapamil hydrochloride peak were well separated from each other. Observed no blank peak interference at the retention time of known peaks as shown in Figure 3. The LC-PDA studies were carried out to check the purity of prototype and each degradation product peak resolved in the UPLC-DAD chromatograms.

Method validation results and tables

System suitability

The criteria of resolution between impurity J and verapamil hydrochloride peak from the system suitability preparation was more than 1.5. RSD for the area of verapamil hydrochloride peak and all the impurities from the replicate injections of standard preparation was less than 10.0%, all the parameters were met during the course of entire validation (Table 1).

Specificity

As shown in the Figure 3, verapamil hydrochloride peak was well separated from each other impurities. No blank peak interference at the retention time of known peaks .The purity angle is less than purity threshold for the verapamil hydrochloride peak in the spiked sample. Hence the method is selective and specific. Figure 4 demonstrates the specificity of the method. Furthermore, specificity of the method was confirmed through forced degradation studies. Verapamil hydrochloride shows degradation products during alkali hydrolysis and oxidation. Since peak purity angle is less than the purity threshold for verapamil hydrochloride peak in all the above degradation samples, the method is stability indicating for the determination of impurities in verapamil hydrochloride. The results from forced degradation studies were summarized in Table 2.

Linearity, limit of detection (LOD) and limit of quantification for related substance method

Linear regression analysis for each ingredient showed that the calibration curveswas linear over the concentration range shown in Table 3. Limit of detection and quantification were also presented in the same table.

Precision-repeatability

RSD for the individual and total impurities were found to be below the acceptance value (Table 4).

Intermediate precision-ruggedness

The RSD of individual and total impurities were calculated and found to be less than 15.0%.The overall RSD between method precision and intermediate precision were less than 15.0%, which demonstrates good precision of the method. Data presented in Table 4.

Accuracy

The recovery of three sample preparations at three different levels were examined, the range was from 89.87% to 114.69%. Results are summarized in Table 4.

Robustness

The results obtained from the robustness study were well within the limit for related substance method (RSD NMT 15.0%). Data incorporated in Table 5.

Solution stability

Cumulative RSD was calculated for the individual impurity and total impurities in the standard solution and was found to be less than 10.0%. Results are summarized in Table 6.

Experimental

System suitability

Standard solution containing verapamil hydrochloride and mixture of impurities at specification limit concentration was injected in six replicate and RSD for the area of all impurities and verapamil hydrochloride peaks were calculated. The resolution between impurity J and verapamil hydrochloride was calculated.

Specificity

During specificity study, verapamil hydrochloride and impurity-A to impurity-P were injected separately. Verapamil hydrochloride sample preparation (1.0mg/mL) spiked with impurities at 1% level (mixture of all impurities at 0.010mg/mL) were also injected. The spectra and purity plots were extracted through diode array detector for each ingredient in the spiked sample. Furthermore, forced degradation studies were conducted in order to prove the stability indicating nature of the method. Sample solution was subjected to acid and base hydrolysis , oxidation using 30% H_2O_2,exposure to white light, UV light(254 nm), humidity(95%) and thermal (105°C). Peak purity was determined using PDA detector.

Linearity, limit of detection (LOD) and limit of quantitation (LOQ)

Six different concentrations of linearity standard solutions were prepared with verapamil hydrochloride and mixture of impurities from LOQ to 200% of the specification limit concentration. Each linearity standard solution was injected in triplicate and linear regression analysis for each ingredient was performed.
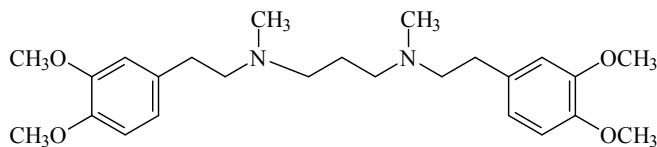
Verapamil hydrochloride	
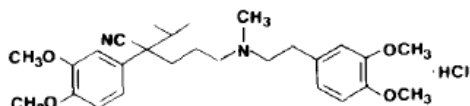
Benzeneacetonitrile, α-[-3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]-3,4 -dimethoxy- α-(1-methylethyl)monohydrochloride
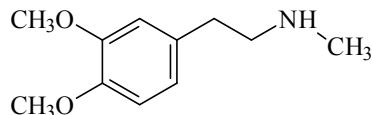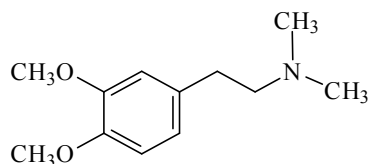
Imp-A

N,N'-bis-[2-(3,4-dimethoxyphenyl)-N,N'-diemthylpropane-1,3-diamine

Imp-B

2-(3,4-Dimethoxyphenyl)-N-methylethanamine

Imp-C

2-(3,4-Dimethoxyphenyl)-N,N-dimethylethanamine

Imp-D
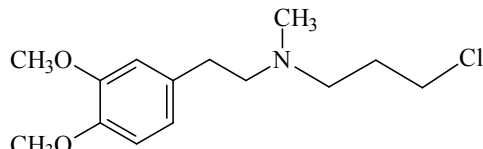
3-Chloro-N-2-(3,4-dimethoxyphenyl)ethyl]-N-methylpropan-1-amine.

Imp-E
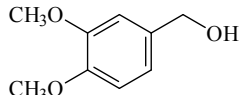
3,4-Dimethoxyphenylmethanol

Imp-F
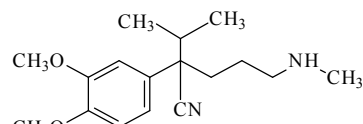
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Imp-G
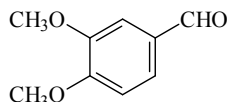
3,4-Dimethoxybenzaldehyde

Imp-H
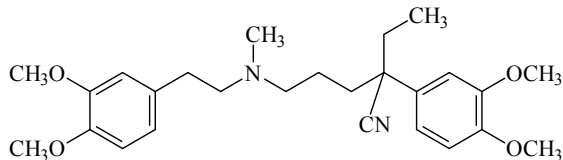
(2RS)-2-(3,4-Dimethoxyphenyl)-5-[[(2-(,4-dimethoxyphenyl)ethyl]methyl)-amino-2-ethylpentanenitrile

Imp-I
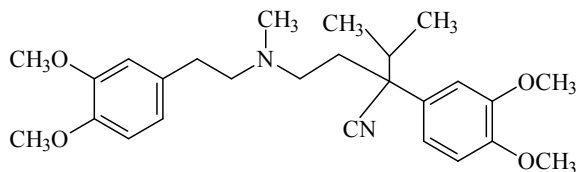
(2RS)-2-(3,4-Dimethoxyphenyl)-2-[2-[[2-(3,4-dimethoxyphenyl)ethyl] (methyl)amino]ethyl]-3-methylbutanenitrile

Imp-J
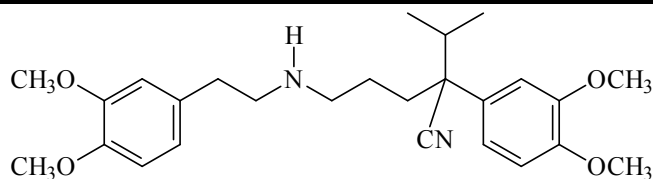
Imp-K
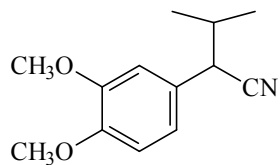
(2RS)-2-(3,4-Dimethoxyphenyl)- 3-methylbutanenitrile

Imp-L
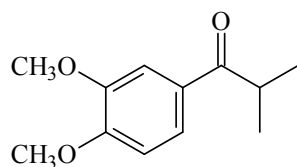
1-(3,4-Dimethoxyphenyl)- 2-methypropan-1-one

Imp-M
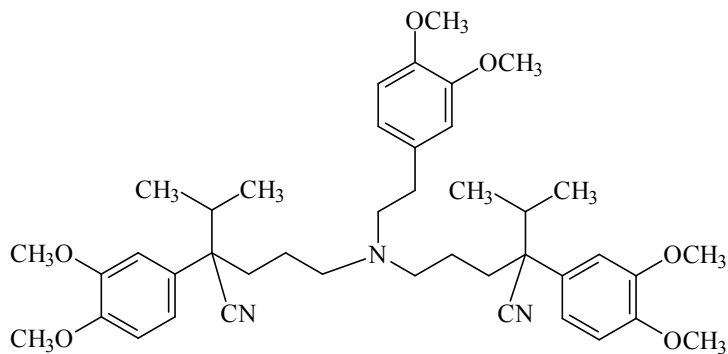
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Imp-N
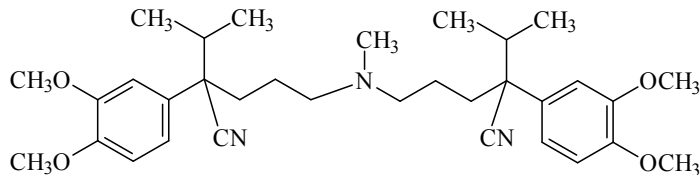
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Imp-O
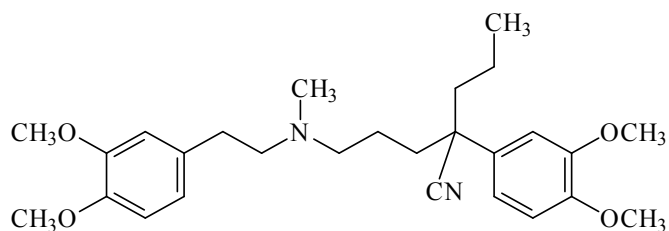
(2RS)-2-(3,4-Dimethoxyphenyl)-5-[2-[2-(3,4-dimethoxyphenyl)ethyl] methyl)amino]-2-propylpentanenitrile

Imp-P
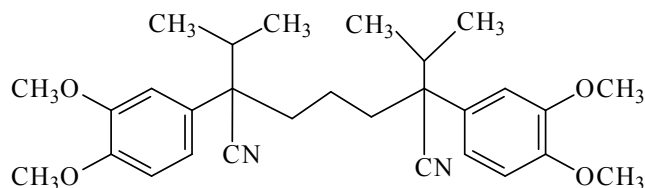
2,6-Bis(3,4-dimethoxyphenyl)-2,6-bis(1-methylethyl)-heptane-1,7-dinitrile

Figure 1. Structure of verapamil hydrochloride and its impurities

Mobile phase-A: 0.04m KH₂PO4 in water pH 7.20
Mobile phase-B: Acetonitrile.
Column : Discovery RP Amide C16 (250x 4.6mm,5µ)
Initial B conc 37%, increased to 65% in 22.0 min, maintained up to 35.0 min with 65%. Revert back to initial conc at 36.0 min and maintained for 14.0 min.
Column flow : 1.5mL/min,
10.00µL injection volume.
Result: Poor resolution between the impurities.

Mobile phase-A: 0.01m Potassium dihydrogen Phosphate pH 2.0 with OPA
Mobile phase-B: Acetonitrile
Column : Acquity UPLC BEH C18 (100x 2.1mm,1.7µ)
Initial B conc 10%, increased   to 70% in 8.0 min, maintained up to 12.0 min with 70%. Revert back to initial conc at 12.10 min and maintained for 2.9 min.
Column flow : 0.5mL/min,
Column temp at 40°C and 1.00µL injection volume.
Result: Poor resolution between impurity J and verapamil.

Mobilephase-A:.10mM Ammonium formate +0.1% OPA in water
Mobilephase-B:0.1%OPAin (water: Acetonitrile)(70:30)
Column : Capcell pak C18(50x 2.0mm,2.0µ)
Initial B conc 10% to 50% in 5.0 min, further to 70% at 8.0 min, further to 90% in 10.0 and maintained at 90% up to 12.0 min, Revert back to initial conc at 13.0 min and maintained for 2.0 min.
Column flow : 0.3mL/min,
Column temp as 40°C and 1.00µL injection volume.
Result: Poor resolution between impurity J and verapamil.

Mobilephase-A:.10mM Ammonium formate +0.1% OPA in water
Mobilephase-B:0.1%OPAin (water :Acetonitrile)(70:30)
Column :Shimpack XR-ODS (75 x 3.00mm, 1.7µ)
Initial B conc 0% to 40% in 8.0 min, further to 70% at 11.0 min, further to 90% 50 15.0 min and maintained at 90% up to 17.0 min, Revert back to initial conc at 18.0 min and maintained for 2.0 min.
Column flow : 0.5mL/min,
Column temp as 50°C and 1.00µL injection volume.
Result: All the peaks are well resolved from each other.

Figure 2. Various trials and conditions for method development

Figure 3. Chromatographic separation of the verapamil hydrochloride and its impurities

Table 1. System Suitability Data

a) %RSD for the area of verapamil and impurities in related substance validation

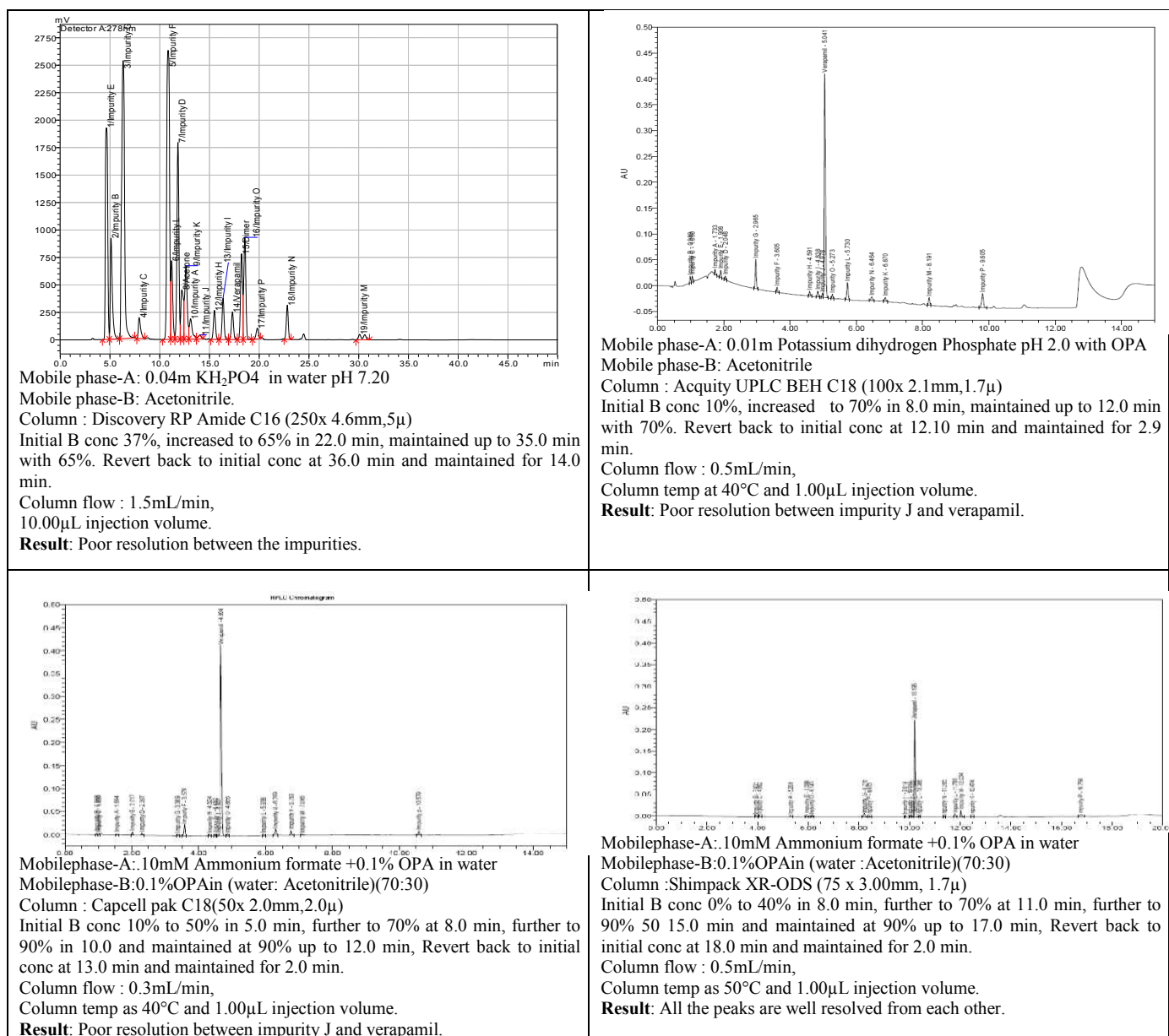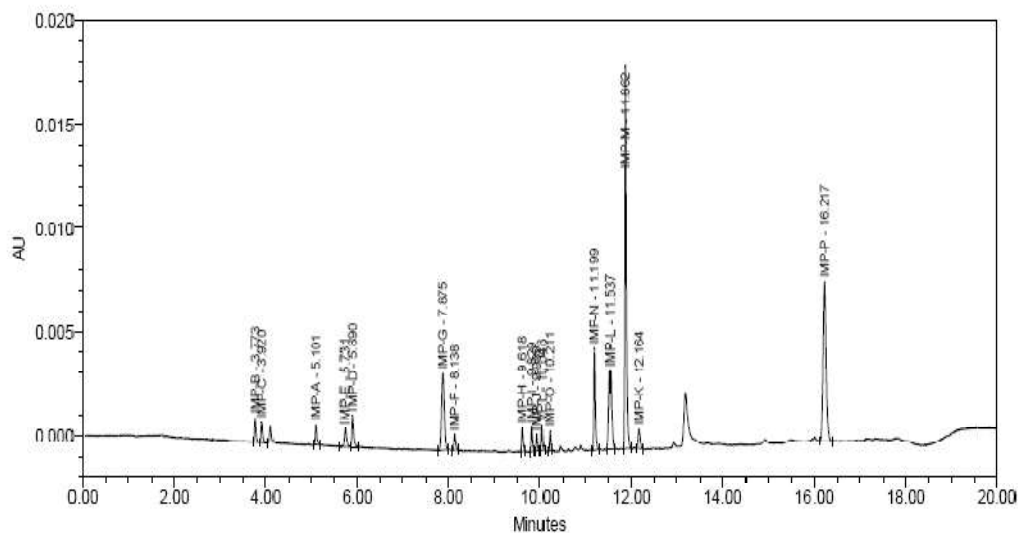| Parameters	Verapamil	Impurities															
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Forced degradation	3.5	5.2	2.2	9.8	2.0	5.0	7.0	0.9	5.3	3.1	9.1	4.1	1.0	1.8	1.7	3.8	3.5
Repeatability	3.5	4.3	3.2	1.2	4.6	3.6	4.5	2.0	4.1	3.2	3.9	4.2	0.9	2.3	4.0	4.6	1.8
Linearity	5.1	3.6	3.3	0.9	3.3	2.1	9.0	1.5	2.3	2.7	7.0	9.4	3.3	6.6	2.7	8.4	2.4
Accuracy	3.0	9.4	3.5	2.9	6.4	3.0	4.8	0.8	2.2	2.2	4.4	2.6	0.5	0.9	1.8	2.1	2.1
Ruggedness	4.5	4.9	2.4	3.4	3.0	2.2	3.3	2.3	3.9	4.1	7.1	4.8	4.9	1.9	2.5	6.2	4.4
Robustness	5.7	9.6	3.8	3.2	4.7	6.5	6.3	1.6	3.8	6.0	2.2	2.2	1.8	0.5	4.8	4.8	4.7

b) Resolution between impurity-J and verapamil

Parameters	Impurity-J and Verapamil
Repeatability	2.0
Linearity	1.9
Robustness	1.9
Solution stability	2.0
Accuracy	2.0
Ruggedness	1.7

Table 2. Result of forced degradation

Control sample (No treatment)			Peak purity	
			Purity angle	Purity Threshold
			0.603	1.790
	Stress Study			
Samples	Condition	% Degradation	Peak Purity	
			Purity angle	Purity Threshold
Acid Degradation	5ml 10N.HCl/ 100°C/30mins	8	0.161	1.899
Alkali Degradation	5ml 10N NaOH/100°C/30 mins	15	0.193	1.829
Peroxide Degradation	5ml 30% H_2O_2/ 100°C/30 mins	18	2.727	3.808
Thermal Degradation	105°C/72Hrs	0	0.611	1.812
Humidity Degradation	25°C/95%RH/72Hrs	0	0.649	1.693
UV light solid (Shorter wavelength)	72 Hrs	-	0.602	1.730
UV light Solution (Shorter wavelength)	72 Hrs	-	0.586	1.574
White light - Solid	72 Hrs	-	0.601	1.897
White light - Solution	72 Hrs	-	0.638	1.818

Table 3. Linearity, Limit of detection (LOD) and Limit of quantification (LOQ)

Component	Concentration range (µg/mL)	Regression equation	R^2	LOQ (µg/mL)	LOD(µg/mL)
Verapamil	0.107-1.984	y = 3067x + 437	0.99235	0.107	0.032
Impurity-A	0.160-2.006	y = 2820x-168	0.99415	0.160	0.048
Impurity-B	0.120-1.983	y = 2807x+156	0.99796	0.120	0.036
Impurity-C	0.100-2.004	y = 2402x+71	0.99562	0.100	0.030
Impurity-D	0.078-1.996	y = 5319x+178	0.99772	0.078	0.024
Impurity-E	0.168-1.996	y = 2689x+178	0.99768	0.168	0.051
Impurity-F	0.210-1.988	y = 2332x-51	0.99656	0.210	0.064
Impurity-G	0.219-2.004	y=16129x-197	0.99932	0.219	0.066
Impurity-H	0.131-1.998	y=2795x+61	0.99896	0.131	0.040
Impurity-I	0.130-1.984	y=2698x+63	0.99928	0.130	0.039
Impurity-J	0.179-1.982	y=1858x+55	0.99984	0.179	0.054
Impurity-K	0.161-1.992	y=3528x-88	0.99938	0.161	0.049
Impurity-L	0.041-1.986	y=13396x-384	0.99826	0.041	0.012
Impurity-M	0.027-1.986	y=13453x-216	0.99730	0.027	0.008
Impurity-N	0.026-2.008	y=11262x-1239	0.99142	0.026	0.008
Impurity-O	0.151-2.022	y=2283x+54	0.99854	0.151	0.046
Impurity-P	0.230-2.026	y=3866x+186	0.99144	0.230	0.070

Linearity results (n=3), Acceptance criteria $R^2 > 0.98$

Table 4. Precision and Accuracy results

Validation step	Parameter	Impurities																Total impurities
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
Method precision	RSD	-	-	-	-	-	-	-	0.04	-	0.05	-	-	-	-	-	-	0.09
Intermediate precision	RSD	-	-	-	-	-	-	-	0.05	-	0.06	-	-	-	-	-	-	0.11
Accuracy (50%, 100% & 120%)	Average (% recovery)	112.3	112.4	98.4	113.4	110.4	95.8	98.4	96.9	96.1	98.7	114.7	89.9	92.4	100.4	104.2	104.7	-
	RSD (% recovery)	3.80	4.51	1.98	3.60	3.49	3.07	3.84	2.01	6.10	6.86	4.57	5.03	7.17	5.03	2.61	7.51	-

Table 5. Robustness

Parameter	Content of related impurities		Total impurities
	Impurity H	Impurity J	
Overall RSD for individual and total impurities values	9.7	9.2	9.4

Table 6. Solution stability (stored at 5°C±2°C)

Parameter		Area of Verapamil and its Impurities																
		Verapamil	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Standard Solution stability	Cumulative RSD between initial to 24hrs	3.12	7.20	1.48	2.82	4.27	4.26	6.01	2.64	3.85	3.60	4.16	6.36	1.70	1.98	1.22	7.04	1.00
Sample solution stability	Cumulative RSD between initial to 24hrs	-	-	-	-	-	-	-	-	0.04	-	0.05	-	-	-	-	-	-

System precision-repeatability-standard solution

The system precision was examined by analyzing standard solution containing verapamil hydrochloride and its impurities at 0.001 mg/mL concentration in six replicates.

Method precision-repeatability-sample solution

Method precision was examined by analyzing verapamil hydrochloride sample in six preparations and calculated the RSD for the individual and total impurities value.

Ruggedness- intermediate precision

Precision was repeated using different analyst, on different day, on different instrument and using column of different lot. Over all RSD was calculated for the individual and total impurities values.

Accuracy

Triplicate sample preparation of verapamil hydrochloride spiked with impurities at 50% level, 100% level and 120% level were analysed.

Robustness

Several below parameters of the method were purposely altered in order to determine the robustness of the method. Standard solution containing verapamil hydrochloride and mixture of impurities at specification limit concentration was injected in six replicate and RSD for the area of all impurities and verapamil hydrochloride peaks was found to be less than 10.0%. The resolution between impurity J and verapamil hydrochloride was found to be more than 1.5.

i) Variation in flow rate ± 10%
ii) Variation in column oven temperature ± 5ºC
iii) Variation in wavelength ± 5 nm
iv) Variation in initial mobile phase B concentration ±10%

Solution stability

Standard solution and sample solution were injected at different time intervals for about 24Hrs kept at 5±2°C. The cumulative RSD was calculated for the area of impurities and verapamil hydrochloride peak in the standard solution and area for individual and total impurities in sample solution.

Conclusion

The UPLC method developed for the determination of impurities in verapamil hydrochloride an active pharmaceutical ingredient is precise, accurate and specific. The method has been validated and satisfactory results were observed for all the tested validation parameters. The developed method can be conveniently used for determining the quality of verapamil hydrochloride in bulk pharmaceuticals. The lower solvent consumption due to short analytical run time of 18.0 min leads to cost effective chromatographic method and greener chemistry.

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