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RESEARCH ARTICLE OPEN ACCESS

RP-HPLC METHOD DEVELOPMENT & VALIDATION FOR CARVEDILOL, IVABRADINE HYDROCHLORIDE DRUG IN BULK & IT'S PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, sensitive, accurate, cost-effective, and stability-indicating HPLC method was developed and validated for the simultaneous estimation of Carvediloland Ivabradine hydrochloride in bulk drugs and combined dosage forms. The method used a Symmetry VL-G4288C UV detector (5 µm) at 265 nm with a 20 µL injection. Carvedilol and Ivabradine hydrochloride showed retention times of 6.973 and 3.876 minutes. It was linear from 1 µg/mL to 12.5 mg/mL for Carvedilol and from 5 mg/mL for Ivabradine, with a detection limit of 0.1 µg/mL for both. Validation confirmed accuracy, precision, and stability, with samples stable for 40 hours and standards for 42 hours.

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INTRODUCTION

Carvedilol(±)-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl] [2-(2-methoxyphenoxy) ethyl] amine,[1] lowers heart rate by blocking beta-adrenoceptors and reduces blood pressure by relaxing vascular smooth muscles through alpha-1 receptor blockade.[2] At higher doses, it also blocks calcium channels and acts as an antioxidant, preventing oxidation of low-density lipoprotein (LDL) and its uptake into coronary arteries.[3]Carvedilol is a medicine that blocks beta receptors, which helps slow down a fast heartbeat caused by exercise. It also blocks alpha-1 receptors, which relaxes blood vessels.[4] This lowers resistance in the blood vessels and reduces blood pressure overall.[5] Ivabradinehydrochloride,3-[3-({[(7S)-3,4-dimethoxybicyclo [4.2.0] octa-1,3,5-trien 7yl]methyl} (methyl)amino)propyl]-7,8-dimethoxy-2,3,4,5-tetrahydro-1H-3-benza-epin-2one,[6] lowers heart rate by selectively inhibiting If ("funny") channels in the sinoatrial node.[7] This slows pacemaker activity, prolongs diastolic depolarization, reduces myocardial oxygen demand, and improves oxygen supply, which helps reduce angina and increase exercise capacity.[8]Carvedilol and Ivabradine are soluble in organic solvents such as ethanol, DMSO, and dimethylformamide (DMF).[9] Analysis is a key part of developing any drug.[10] There must be a reliable and tested method to measure the drug in its raw form, in drug delivery systems, during dissolution studies, and in biological samples.[11]

If no such method exists, it is important to create one that is simple, sensitive, accurate, precise, and reproducible.

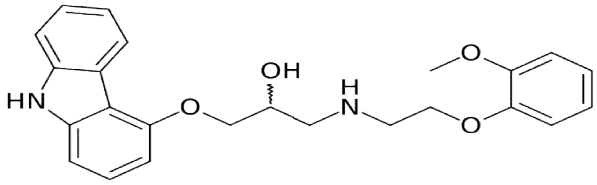

Figure 1. Structure of Carvedilol

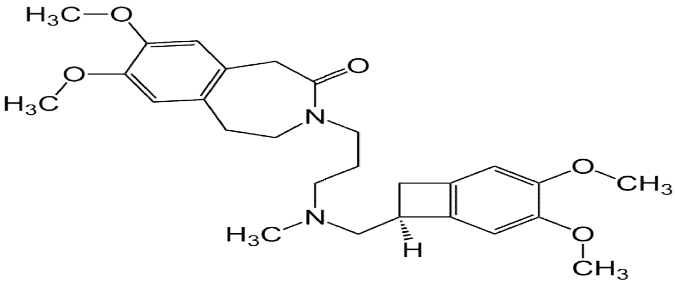

Figure 2. Structure of Ivabradine

MATERIALS AND METHODS

Chemicals and Reagents: All reagents and chemicals used were of AR grade and HPLC grade Carvedilol, Ivabradine hydrochloride reference standard were standardizedagainst IP reference standard.[12] Tablets named Cardivas IN was purchase from local market. Acetonitrile, Methanol, Ortho-phosphoric acid and Potassium dihydrogenphosphate anhydrous AR (Merck Ltd., India), were used.

Instrumentation and Chromatographic Condition: The following chromatographic conditions were established by trial and error and were kept constant throughout method.Chromatographic system equipped with Quaternary Gradient pump, Waters alliance HPLC system 1220 series infinity LC gradient system VL - G4288C UV Detector fitted with Symmetry C18 (75 x 4.6 x 5µm) column.Empower 3.8.0 software was use to interpretation of chromatograms. The mobile phase degassed by sonication using ultrasonic bath (Labman LMUC2). Analytical balance Phoenix Gold (Model No. 300 P) used for weighing standard and sample. Equip-tronich (Model No.EQ-610) pH meter used to measure pH of the mobile phase. The experimentperformed utilizing 5µm column of Symmetry C18 (75 mmx 4.6 mm) at a flow rate of 1.0 ml/ min. Ambient the columntemperature. Chromatography runs with 10µl injectionvolume at 265 nm detection wavelength. Chromatography runs for 4 minutes.

Preparation of Diluent

Standard Solution: Accurately weighed quantity 10 mg of Carvedilol (CRV) &Ivabradine (IVB) was dissolved in mobile phase and volume was made up to 100 ml mark. The stock standard solution was diluted further with mobile phase to get final concentration of about 12.5 mg/ml of CRV and 5 mg/ml of IVB.

Sample Solution: The twenty tablets were weighed, and then average weight was determined and finely grounded. The weight of the powdered tablet equivalent to 12.5 mg of CRV and 5 mg of IVB were transferred into a 100 mL standard volumetric flask. Added 50 mL of solvent sonicated for 10 min and diluted to 100 mL with the same solvent and then filtered through Whatmann filter paper No: 41.

Method Validation

Harmonization (ICH): The proposed method has been verified to meet the requirements of the International Conference on Harmonization (ICH).

System Suitability: System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.[13] The tests were performed by collecting data from five replicate injections of standard solutions.

Method Validation

Specificity: Peak purity profiling studies used to determine the method's specificity. The purity of the drug was determined by examining the spectrum at the beginning, middle, and end of the peak. Using software, the peak purity was determined for both standard solution and sample solution. Further, theinterference of diluent and excipient peak was monitor.[14]

Linearity: According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration.[15] The chromatograms of the resulting solutions were recorded. CRV and IVB marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug.

Accuracy: It was ascertained on the basis of recovery studies performed by standard addition method.

RESULTS & DISCUSSIONS

FT-IR analysis: The IR spectroscopy theory utilizes the concept that molecules tend to absorb specific frequencies of light that are characteristic of the corresponding structure of the molecules.[16] The FTIR spectrum of CRV showed a distinct peak at 3729.05 cm^{-1} (O–H stretching vibrations), 2897.60 cm^{-1} (C-H stretching vibrations), 1652.78 cm^{-1} (C=O Stretching), 1545.52 cm^{-1} (N-H Bending), 1381.10 cm^{-1} (C-H bending medium) and 1022. The FTIR spectrum of IVB showed a distinct peak at 3407.22 cm^{-1} (amine stretching vibrations), 1694.87 cm^{-1} (tri substituted alkene), 1825.81 cm^{-1} (C=O Stretching), 1284.53cm^{-1} (aromatic amine), 1524.84 cm^{-1} (C-N stretch medium) and 1071.87.This FTIR spectra confirmed the drug.

UV Spectroscopy Analysis: The ultraviolet absorption spectrum of CRV & IVB was obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm. The wavelength maxima (λmax) were analyzed.[17]

Selection of mobile phase: Each mobile phase was filtered through Whatman filter paper No. 42. Peak, well resolved peaks with symmetry within limits and significant Based on sample solubility & stability, various mobile phase compositions were evaluated to achieve acceptable separation using selected chromatographic conditions.[18] From various mobile phases tried, mobile phase containing Acetonitrile: Phosphate buffer (60:40) pH 3 by OPA was selected, since it gives sharp reproducible retention time for CRV & IVB. Fig.No.03-04.

Preparation of calibration curve: The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from µg/ml for 2-20 µg/ml CRV and 2-20 µg/ml for IVB drug solutions were injected and peak area was recorded. The graph plotted as the concentration of the drug Vs peak area depicted in Fig. No.05 and 06.

System suitability test: System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions Tableno.01.

Application of proposed method for estimation of CRV and IVBLaboratory mixture: The peak area of standard laboratory mixture and sample laboratory mixture was compared to obtain the concentration.Table No 02.

Application of proposed method for estimation of CRV and IVB in formulation: Equal volume (20µL) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. Table No.03 The content of CRV and IVB was calculated by comparing a sample peak with that of standard. Fig.No.07.

Validation parameters:

a) Accuracy:

- It was ascertained on the basis of recovery studies performed by standard addition method.
- The results of recovery studies and statistical data are recorded in Table No.04.

b) Precision:

- Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.Table no.5.

c) Ruggedness:

The studies of ruggedness were carried out fewer than two different conditions-

1) Days
2) Analyst.

i) **Interday (Different days):** Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 06

ii) **Intraday:** It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The percent label claim was calculated using formula & Result and statistical data are shown in Table No.07.

iii) **Different analyst:** The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

d) Specificity: Specificity was measured as ability of the proposed method to obtain well separated peak for CRV and IVB without any interference from component of matrix.

The Retention time for –
CRV – 6.973
IVB – 3.876

The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.Typical chromatogram is shown in the Fig. No. 07.

e) Linearity and range: According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. CRV and IVB marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug.The plot showing linearity and range study for CRV and IVB is shown in the Fig. No. 07and 08.

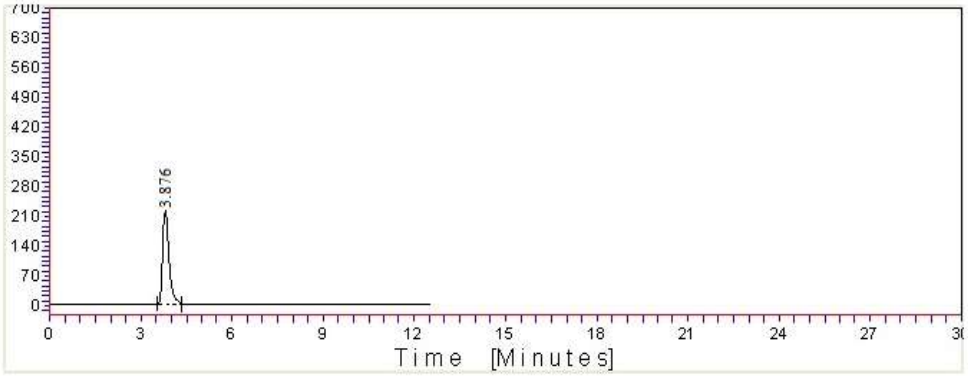

Fig. 3. Final Chromatogram obtained by using Acetonitrile: Phosphate buffer 5mm (60:40) pH 3 as mobile phase

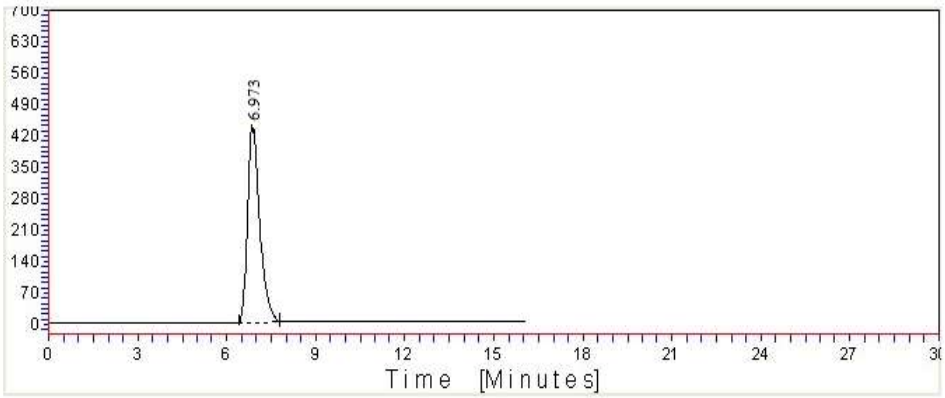

Fig. 4. Final Chromatogram obtained by Acetonitrile: Phosphate buffer 5 mm (60:40) pH 3 as mobile phase of IVB

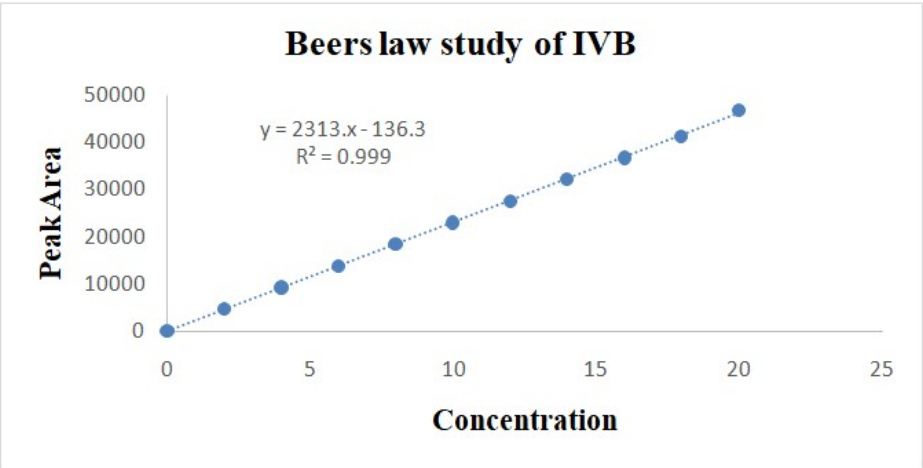

Fig. 5. Standard calibration curve for CRV

f) Robustness: Robustness is a measure of how well an analytical method can produce reliable results when there are small changes to the experimental conditions. The robustness study for CRV and IVB shown in following Table No.08.

g) Limit of Detection (LOD) and Limit of Quantitation (LOQ): Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

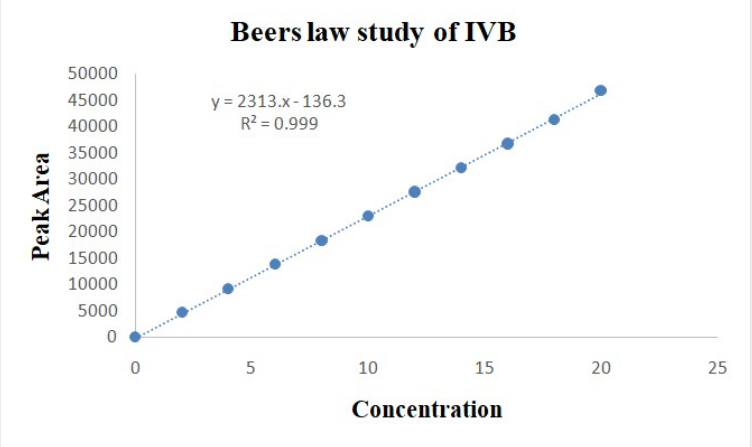

Fig. 6. Standard calibration curve for IVB

Table 1. Result of System Suitability Study

Sr. No.	Peak area		Retention Time		Asymmetry		Efficiency	
	CRV	IVB	CRV	IVB	CRV	IVB	CRV	IVB
1	52161.9	11456.2	6.973	3.876	0.713	0.253	156212.2	58976.5
2	52172.3	11442.5	6.971	3.877	0.718	0.256	156215.5	58978.4
3	52167.1	11448.2	6.969	3.881	0.718	0.252	156234.2	58972.6
4	52146.2	11456.1	6.968	3.872	0.719	0.258	156221.6	58979.4
5	52161.9	11450.5	6.974	3.876	0.721	0.256	156212.2	58978.7
Mean	52161.88	11450.7	6.971	3.8764	0.7178	0.255	156219.14	58977.12
± S.D	9.7684185	5.764980	0.002549	0.00320	0.00294	0.00244	9.2524591	2.7453597
C.V	0.0001872	0.000503	0.000365	0.00082	0.00410	0.00960	0.0000592	0.0000465

Table 2. Results and statistical data for estimation of CRV and IVB in lab. Mix

Sr. No.	Weight of std. (mg)		Weight of sample (mg)		Peak area of std		Peak area of sample		% Drug estimation	
	CRV	IVB	CRV	IVB	CRV	IVB	CRV	IVB	CRV	IVB
1.	10	10	10	10	52167.1	11456.8	52062.8	11468.3	99.8	100.1
2.			9.9	10			52010.6	11411.0	99.7	99.6
3.			10	10.1			51958.4	11491.2	99.6	100.3
								Mean	99.70	100.00
								±S.D.	0.100	0.361
								C.V.	0.001	0.004

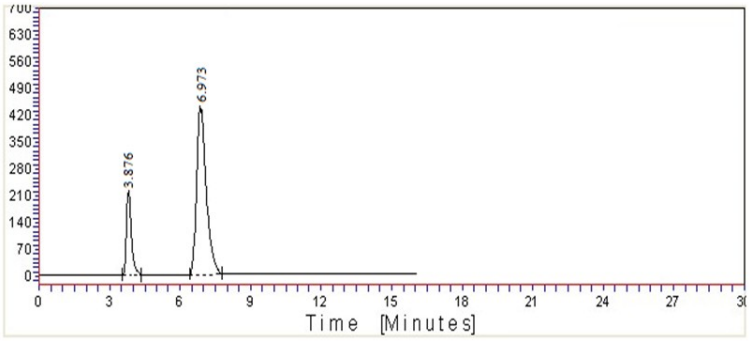

Fig. 7. Chromatogram obtained by formulation of CRV and IVB

Table 3. Results and statistical data for estimation of CRV and IVB in marketed formulation

Sr. No.	Weight of std.(mg)		Weight of sample (mg)	Peak area of std.		Peak area of sample		% Label claim	
	CRV	IVB		CRV	IVB	CRV	IVB	CRV	IVB
1.	10	10	115	52167.1	11456.8	52219.3	11491.2	100.1	100.3
2.			115			52271.4	11502.6	100.2	100.4
3.			115			52114.9	11388.1	99.9	99.4
							Mean	100.07	100.03
							±S.D.	0.153	0.551
							C.V.	0.002	0.006

*Results are mean of three replicates

Table 4. Results and statistical data for Recovery study of CRV and IVB

Sr. No.	wt. of formul ation (mg)	Amount of Drug Added in (µg/ml).		Peak Area of stand.		Peak Area of sample		% Recovery	
		CRV	IVB	CRV	IVB	CRV	IVB	CRV	IVB
1	115	1	1	52167.1	11456.8	52323.6	11445.3	100.3	99.9
2		1	1			52375.8	11468.3	100.4	100.1
3		1	1			52427.9	11411.0	100.5	99.6
4		2	2			52688.8	11491.2	101	100.3
5		2	2			52740.9	11502.6	101.1	100.4
6		2	2			51801.9	11388.1	99.3	99.4
7		3	3			52480.1	11433.9	100.6	99.8
8		3	3			52584.4	11422.4	100.8	99.7
9		3	3			52636.6	11411.0	100.9	99.6
						Mean		100.54	99.87
						±S.D.		0.541	0.339
						C.V		0.005	0.003

Table 5. Results and statistical data of Precision Study

Brand Name: Cardivas IN

Sr. No	Weight of std.(mg)		Wt. of sample (mg)	Peak area of Std.		Peak area of sample		% Label claim	
	CRV	IVB		CRV	IVB	CRV	IVB	CRV	IVB
1.	10	10	115	52167.1	11456.8	52427.9	11411.0	100.5	99.6
2.			115.1			52688.8	11548.5	101	100.8
3.			115			52740.9	11559.9	101.1	100.9
							Mean	100.87	100.43
							±S.D.	0.321	0.723
							C.V.	0.003	0.007

Table 6. Results and statistical data of Interday Study

Brand Name: Cardivas IN

Sr. No.	Weight of std.(mg)		Wt. of sample (mg)	Peak area of std.		Peak area of sample		% Label claim	
	CRV	IVB		CRV	IVB	CRV	IVB	CRV	IVB
1.	10	10	115	52167.1	11456.8	51801.9	11548.5	99.3	100.8
2.			115			52480.1	11559.9	100.6	100.9
3.			115			52584.4	11582.8	100.8	101.1
							Mean	100.23	100.93
							±S.D.	0.814	0.153
							C.V.	0.008	0.002

*Results are mean of three replicates

Table 7. Results and statistical data of Intraday Study

Brand Name: Cardivas IN

Sr.No.	Weight of sample (mg)	Peak area of std.		Peak area of sample		% Label claim	
		CRV	IVB	CRV	IVB	CRV	IVB
1.	115.1	52167.1	11456.8	52323.6	11433.9	100.3	99.8
2.	115			52375.8	11422.4	100.4	99.7
3.	115			52427.9	11411.0	100.5	99.6
					Mean	100.40	99.70
					±S.D.	0.100	0.100
					C.V.	0.001	0.001

*Results are mean of three replicates

Table 8. Result of Robustness study of CRV and IVB

Sr. No.	Condition	Parameter	CRV Peak Area	CRV RT	IVB Peak Area	IVB RT
01	Change of wavelength	263 nm	52161.9	6.971	11455.7	3.877
		265 nm	52172.3	6.973	11457.9	3.876
		267 nm	52167.1	6.976	11456.8	3.879
02	Change in Temperature	30 °C	52146.2	6.972	11452.2	3.875
		25 °C	52161.9	6.973	11455.7	3.876
		20 °C	52104.5	6.973	11443.1	3.871
03	Change in Flow Rate	0.8 ml/min	52130.6	6.984	11448.8	3.886
		1 ml/min	52166.6	6.975	11456.7	3.876
		1.2 ml/min	52141.0	6.961	11451.1	3.865
04	Change in Mobile Phase	65:35	52219.3	6.969	11468.3	3.869
		60:40	52167.1	6.973	11456.8	3.876
		55:45	52166.6	6.977	11456.7	3.881

Table 9. LOD & LOQ of CRV & IVB

Sr. No.	Drug Name	LOD µg/ml	LOQ µg/ml
1	CRV	1.15	2.67
2	IVB	0.88	1.98

Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision accuracy. The result shown in following Table 09.

CONCLUSION

A reliable, fast, cost-effective, sensitive, and stability-indicating method was developed and fully validated to measure Carvedilol and Ivabradinehydrochloride in-process samples and Cardivas IN tablets. Following ICH guidelines, the method was created using RP-HPLC and carefully checked.The validation covered system suitability, specificity, precision, ruggedness, accuracy, linearity, robustness, detection and quantification limits, and solution stability. This method can measure both drugs at levels as low as 0.5 ppm. It can be used for both initial testing and stability studies in pharmaceutical manufacturing.

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