Article

Preparative Isolation and High-Resolution Mass Identification of 10 Stressed Study Degradants of Nicorandil Tablets

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Nicorandil is a potent drug with a dual mechanism of action which results in its wide application in treating angina patients. Since the literature review showed no single preparative method for isolation and liquid chromatography−mass spectrometry (LC−MS) compatible [high-performance liquid chromatography (HPLC) or ultra performance liquid chromatography (UPLC)] method for identification for the impurities generated in process and by force degradation was reported. Thus an attempt was made to develop a single preparative method for isolation and HPLC and UPLC methods for identification of impurities of nicorandil in tablet dosage form. Ten degradants were found in the formulated drug under the stress conditions, [40°C/ 75% relative humidity for 2 months] in an LC−MS compatible method. A cost effective and high-throughput simple gradient preparative HPLC method was developed with a runtime of 30 min to isolate all the degradants. The method is capable and can be used to isolate further degradants. To detect these degradants, rapid and effecient HPLC and UPLC methods were developed and the same were identified by UPLC-time of flight mass spectrometry (TOF MS). The crux of this work involves a single preparative isolation method and UPLC identification method for all degradants with a very short runtime, i.e., 13.8 min, and, furthermore, the method has the potential to separate a wide range of degradants.

Introduction

Nicorandil (2-[(pyridin-3-ylcarbonyl) amino] ethyl nitrate) (Figure 1) is a nicotinamide derivative and has been developed as an anti-anginal medication. Nicorandil belongs to the class of compounds known as potassium channel activators, which are characterized by their arterial vasodilator properties (1−5). In addition, nicorandil has venodilating properties that are attributable to a nitrate group in its chemical structure. Therefore, combining these two vasodilator mechanisms, nicorandil represents a novel type of compound for use in the treatment of angina pectoris (6−8). Nicorandil is a drug that has been developed as an anti-anginal medication. Its structure (Figure 1) is characterized by a dual mechanism of action (9, 10). The nicotinamide moiety acts as an opener of ATP-sensitive potassium channels, whereas the NO_2 group explains its nitrate-like properties. The NO-like action leads to a dilation of the large coronary arteries, whereas its potassium channel opening action is responsible for dilation of coronary resistance vessels. Nicorandil may be effective even in patients with rest/effort angina who do not respond to combination therapy with calcium antagonists and oral nitrates (11, 12).

In general, solid active pharmaceutical ingredients (APIs) are formulated with excipients as in tablets and/or capsules. Since the active ingredient interacts with the excipients and the formulated product is stored under different conditions, the study of stability of APIs is critical in the drug development process. Study of stability under stressed conditions is important, since it can cause many degradation reactions. It is mandatory to identify and structurally characterize any impurity formed during production and stability, and exceeding the identification threshold in the drug product (13−19). For low-level impurities or degradation products, this quite often involves the development of a high-performance liquid chromatography (HPLC) method, and isolation and characterization techniques (20−24). A literature search reveals that several analytical methods include HPLC (25−27) and gas chromatography−mass spectrometry (GC−MS) (28) for the estimation of nicorandil from biological fluids and/or pharmaceutical formulations. The stability of nicorandil tablets under stressed conditions is reported by HPLC and GC−MS (29, 30) but not included all degradatns. The monitoring of impurities is important for pharmaceutical drug development and quality control of drugs and their formulation. Hence, the present manuscript deals with the isolation of 10 degradants, obtained by the storage of the tablets under stressed conditions [40°C/75% relative humidity] for 2 months and identification by UPLC-time of flight mass spectrometry (TOF MS).

Experimental

Chemicals and reagents

The investigated samples of nicorandil drug product were obtained from R&D of Dr. Reddy's Laboratories Ltd., Hyderabad, India. HPLC-grade acetonitrile and analytical-grade ammonium acetate were purchased from Merck (Darmstadt, Germany). Ultra-pure water was collected from the TKA Millipore water purification system.

Instrumentation

Reversed-phase−liquid chromatography methods

Samples were analyzed on a Waters (Milford, MA, USA) HPLC system equipped with a 2690/5 Separation Module and 2998 series photodiode array detector. An Inertsil (GL Sciences Inc., Torrance, USA) C8−3,250 × 4.6 mm, 5.0 μm column was used for the chromatographic separation. Mobile phase A consists of 10 mM ammonium acetate buffer and Mobile phase B consists of acetonitrile and the above buffer in the ratio of 90 : 10 (v/v). Optimized gradient conditions for the separation with a timed gradient program of T (min)/%B (v/v) are as follows: 0/2, 10/ 10, 15/25, 25/40, 26/2 and 28/2. Sample detection was monitored at a wavelength of 262 nm. Chromatography was

performed at ambient temperature using a flow rate of 1.0 mL/min and injection volume of 10 μL. On the basis of the above HPLC method, an efficient and short runtime ultra performance liquid chromatography (UPLC) method was developed to identify the impurities by UPLC-TOF MS. Samples were analyzed on Waters (Milford, MA, USA) ACQUITY™ UPLC system. An
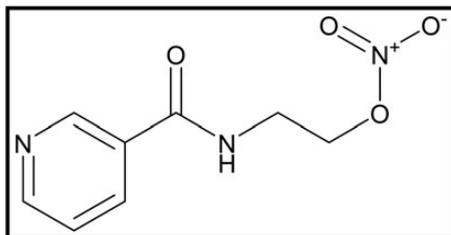

Figure 1. Chemical structure of nicorandil.

ACQUITY UPLC™ BEH C8, 2.1 × 100 mm, 1.7 μm (Waters, Milford, MA, USA) column was used for the chromatographic separation. Mobile phase A consists of 10 mM ammonium acetate buffer and Mobile phase B consists of acetonitrile and the above buffer in the ratio of 90 : 10 (v/v). Optimized gradient conditions for the separation with a timed gradient program of T (min)/%B (v/v) are as follows: 0/2, 3.8/10, 5.8/25, 9.8/35, 11.8/60 and 13.8/2. Sample detection was monitored at a wavelength of 262 nm. Chromatography was performed at ambient temperature using a flow rate of 0.2 mL/min and injection volume of 2.0 μL. Both HPLC and UPLC methods were developed to resolve even further more degradants.

Preparative HPLC method

Degradants were isolated from the sample using an Agilent (CA, USA) 1200 series preparative HPLC system, which was equipped
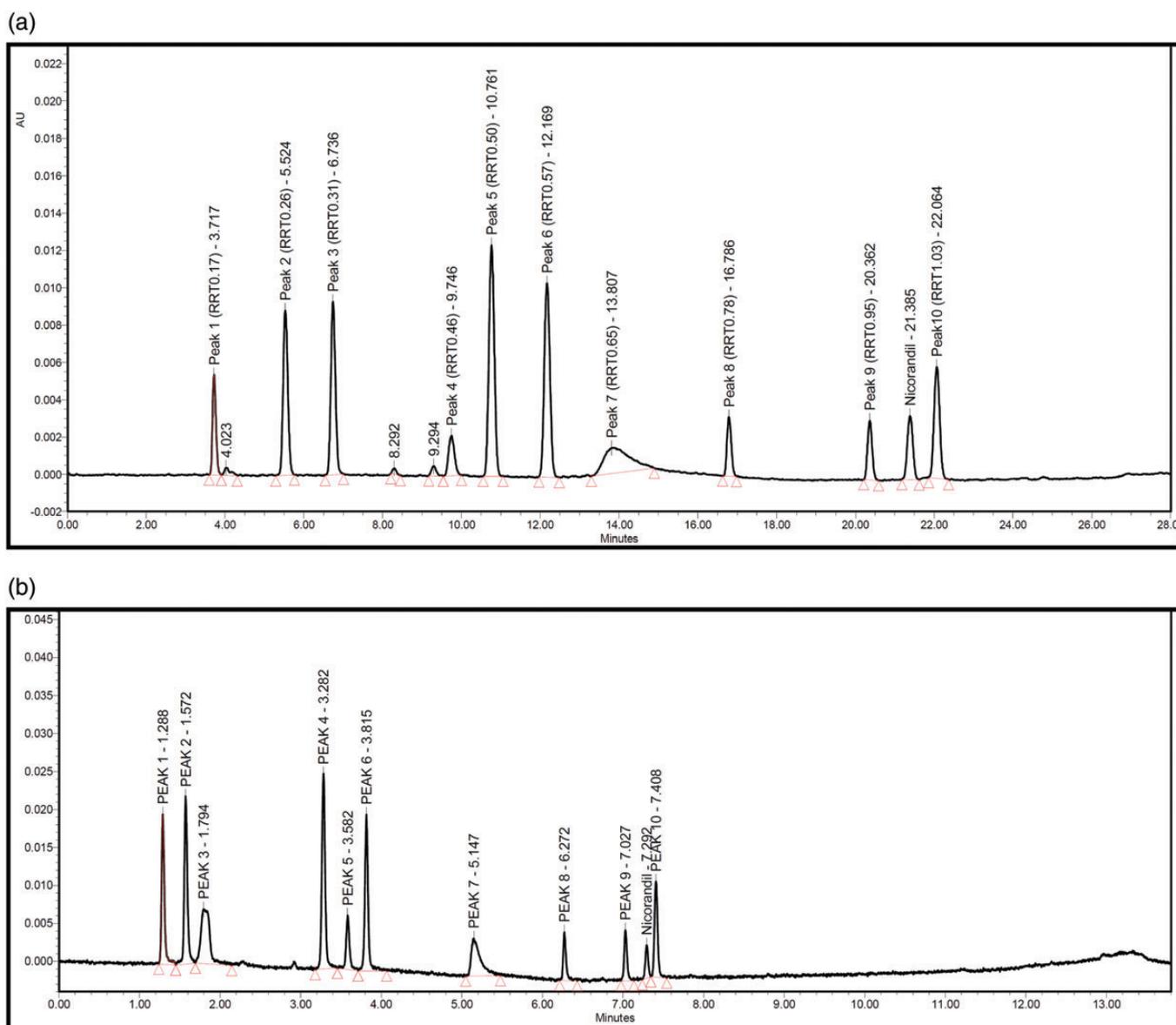
Figure 2. (a) HPLC detection chromatogram of all degradants. (b) UPLC detection chromatogram of all degradants.

with an automated fraction collector and photodiode array detector. The data were collected and processed using the Chemstation software. An aliquot of 20 mg/mL of the sample was prepared to load on to the preparative column. Target impurity fractions were collected from several injections and then pooled. These fractions were concentrated separately by using Rotavapor (Heidolph Laboratory 4002 control, Lübeck, Germany) under high vacuum. The aqueous solutions were subjected to lyophilization to get the impurities in solid state. An Inertsil (GL Sciences Inc.) C18, 250×10 mm, 5 μm column was employed for the separation of all degradants. Mobile phase A consists of 10 mM ammonium acetate buffer and Mobile phase B consists of acetonitrile and the above buffer in the ratio of 90 : 10. Gradient conditions employed for the separation with a timed gradient program of T (min)/%B (v/v) are as follows: 0/5, 5/5, 10/15, 15/25, 25/35, 27/5, 30/5. The flow rate was kept at 5 mL/min. Detection was carried out at 262 nm.

UPLC-TOF MS

The UPLC-TOF MS system consisted of an ACQUITY™ UPLC system and a Micro mass LCT Premier XE Mass Spectrometer (high sensitivity orthogonal time-of-flight instrument, Waters, Millford, MA, USA) equipped with a lock mass sprayer, operating in either the positive or negative ion mode. All analyses were acquired using the lock spray to ensure accuracy and reproducibility; leucine-enkephalin was used as the lock mass.

Sample preparation

Analytical chromatography

The tablet powder equivalent to 10 mg of nicorandil was transferred into a 10 mL volumetric flask, dissolved and diluted to volume with diluent (90 : 10, Water : Acetonitrile), and filtered through 0.45 μm filter to get clear solution.

Preparative chromatography

The tablet powder equivalent to 500 mg of nicorandil was transferred into a 25-mL volumetric flask, dissolved and diluted to volume with analytical diluent and filtered through 0.45 μm filter to get clear solution; then 0.5 mL of the solution was injected into the preparative HPLC system.

UPLC-TOF MS

Sample of concentration 0.02 mg/mL in analytical diluent was infused in TOF-MS at a flow rate of 0.2 mL/min. High-resolution (W mode, FWHM 10500) positive polarity scan responses were collected from m/z 100 to 1000 at a rate of 1.0 s/scan.

Results

Detection of impurities

A typical LC−MS compatible analytical LC chromatogram (Figure 2a and b) of a stability batch of nicorandil drug product was recorded using the HPLC and UPLC methods as described. The UV spectra of all degradants are shown in Figure 3. The LC−MS compatible methods are able to separate all the degradants of described relative retention times (RRTs) (Table I).
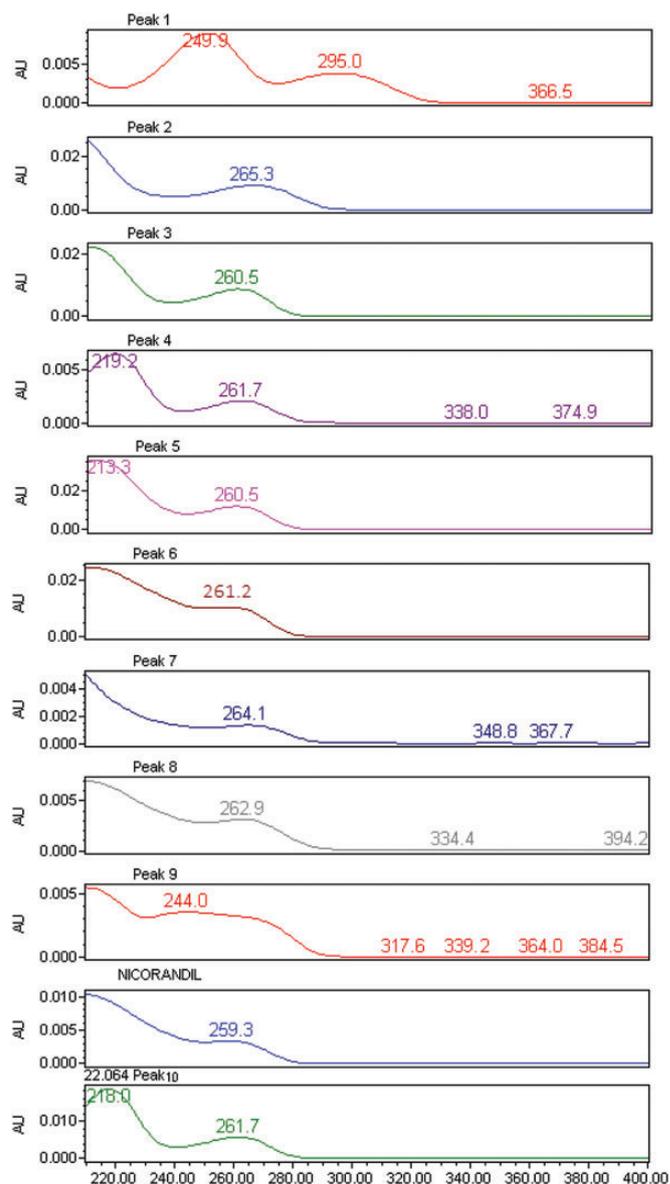

Figure 3. UV spectra of all degradants.

Table I

RRTs in HPLC and UPLC Methods and Purity of All Isolated Impurities

Degradants	RRTs in the HPLC method	RRTs in the UPLC method	Purity of isolated impurities (%)
Peak 1	0.17	0.18	98.2
Peak 2	0.26	0.22	98.0
Peak 3	0.31	0.25	99.2
Peak 4	*0.46*	*0.49*	97.8
Peak 5	*0.50*	*0.45*	97.5
Peak 6	0.57	0.52	98.0
Peak 7	0.65	0.71	97.6
Peak 8	0.78	0.86	99.0
Peak 9	0.95	0.96	98.8
Peak 10	1.03	1.02	98.5

Italic values denote change in the relative retention times.
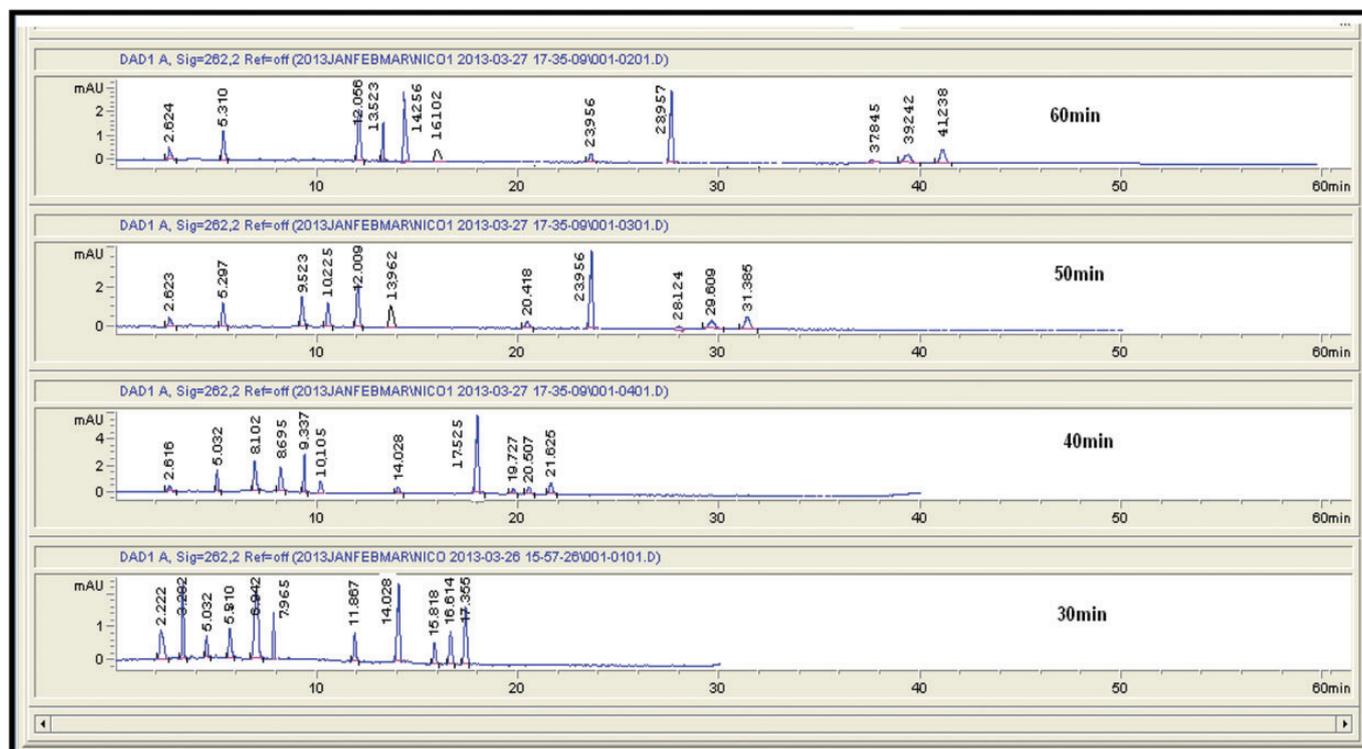
Figure 4. Preparative gradient optimization chromatograms of all degradants.

Preparative HPLC

All the impurities were separated with reasonable resolution in the LC–MS compatible analytical method. The same method was adopted for the preparative separation with necessary modifications and scaled up, facilitating the isolation of all impurities in a single method. Preparative gradient optimization chromatograms were shown in Figure 4. All the impurity fractions were collected separately from 50 injections and pooled together separately. The pooled samples were rota-evaporated separately at 25°C under high vacuum to remove the organic solvents and solidified through the lyophilization process. The purities of isolated impurities are summarized in Table I.

UPLC-TOF MS analysis

UPLC-TOF MS analysis was performed for all degradants. The positive TOF MS + spectrum (protonated molecular ion) of all peaks are shown in Supplementary data, Figure S1. The positive TOF MS + spectrum (protonated molecular ion) of Peak 1 corresponding to the molecular formula $C_8H_{12}N_3O$, calculated (Calcd) for: 166.0979; found: 166.0980, Peak 2 corresponding to molecular formula $C_6H_6NO_2$, Calcd for: 124.0399; found: 124.0399, Peak 3 corresponding to molecular formula $C_6H_6NO_2$, Calcd for: 124.0396; found: 124.0399, Peak 4 corresponding to molecular formula $C_6H_7N_2O$, Calcd for: 123.0577; found: 123.0558, Peak 5 corresponding to molecular formula $C_8H_{11}N_2O_2$, Calcd for: 123.0557; found: 123.0558, Peak 6 corresponding to molecular formula $C_8H_{11}N_2O_2$, Calcd for: 167.0813; found: 167.0821, Peak 7 corresponding to molecular formula $C_{35}H_{24}N_7O_5$, Calcd for: 622.1859; found: 622.1839, Peak 8 corresponding to molecular formula $C_{16}H_{18}N_5O_5$, Calcd for: 360.1299; found: 360.1308, Peak 9 corresponding to molecular formula $C_8H_9N_2O$, Calcd for: 149.0709; found: 149.0715 and Peak 10 corresponding to molecular formula $C_7H_8NO_2$, Calcd for: 138.0551; found: 138.0555.

Discussion

Preparative liquid chromatography is a versatile, robust and widely used dominant purification technique for the isolation of pharmaceutical impurities. The objective of an analytical HPLC run is the qualitative and quantitative determination of a compound. For a preparative HPLC run it is the isolation and purification of a valuable product. Since preparative HPLC is a rather expensive technique compared with traditional purification methods such as distillation, crystallization or extraction, it had been used only for rare or expensive products. With increasing demand for production of highly pure compounds with regard to varying amounts for activity, toxicology and pharmaceutical screening, the field of operation for preparative HPLC is changing. The objective of present work is to isolate the degradants formed during the stability of nicorandil tablets under stressed conditions. The tablets were studied by HPLC and UPLC and it was found that 10 impurities are crossing the identification threshold and warrants not just identification but also subsequent characterization. The present study deals with the isolation of all the 10 degradants in a single preparative HPLC method. All the impurities were separated with reasonable resolution by the LC–MS compatible analytical method. The same method was adopted for the preparative separation with necessary modifications and scaled up, which facilitated the isolation of all impurities in a single method. Scale-up from analytical LC

to preparative-scale chromatography is often time-consuming and wasteful of materials unless an optimized scale-up strategy is employed. The modifications include scale-up of flow, sample loading, injection volume from analytical scale to preparative scale and changes in the gradient program of preparative LC. Several trials were taken to optimize a gradient method with shorter runtime to achieve high throughput. The three important parameters used to judge the result of a preparative run are purity, yield and throughput. Since these parameters are dependent on each other, it is not possible to optimize a preparative HPLC method with respect to all three parameters. An optimized preparative HPLC run is that which compromises all the three parameters. By keeping all these parameters in mind, finally a simple and cost-effective gradient preparative HPLC method was developed and employed to isolate all the degradants with a better resolution and a shorter runtime of 30 min. The peaks are almost baseline-separated, which leads to high purity and yield, and throughput is as high as possible. The purities of isolated impurities were high enough. The isolated impurities were ideintified using a time−of-flight mass analyzer.

Conclusions

To conclude, 10 degradants of nicorandil tablets were found during the accelerated stressed conditions. The impurities were isolated by a single preparative HPLC method and identified by UPLC-TOF MS. There was no literature with regard to the single preparative HPLC method for isolation of all degradants or with regard to LC−MS compatible HPLC and UPLC methods.

Acknowledgments

Supplementary data

Supplementary data are available at *Journal of Chromatographic Science* online.

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